FLUORESCENCE ANALYSIS

in

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EDITORIAL PREFACE.

In these days of intensive and extensive research, every worker in science or its applications knows how rapidly the contents of text-books and encyclopædias become out of date; and those who wish to see new work published know the difficulties which abnormal taxation and high labour costs offer to the realisation of their desire. The one obvious solution of the problem is the publication of monographs that would focus attention upon recent work, or upon new aspects of old work, and upon their theoretical implications. Such books are usually written by experts for other experts in related fields of science, or for the well-educated layman whose thirst for new knowledge has not been quenched by the more sensuous outpourings of the ephemeral press.

It is interesting at times to speculate upon what aspects of our civilisation the future historian will select as the most characteristic of our time. Scientific discoveries and their application to human welfare, we may be sure, will find a place; and to these many will add the growth of our sense of "values." The value of new work in science varies greatly: the golden grain is always accompanied by chaff, and there is no precious ore without country rock. Owing to the difficulty of assessing the value of work at the time of its production, we find that our scientific periodicals stand in danger of being swamped by the mass of second- and third-rate material that is thought to be worth publishing, but which

posterity will decree would have been better left in manuscript form. It is the first duty of the monograph writer to estimate the value, either actual or potential, of recent work upon the subject of which he writes: he must pick out the plums to save others from the indigestion that follows eating the whole pie. Further, in addition to being accurate, his work must be presented in a form that is both assimilable and attractive; in other words, he must show that lucid exposition can be achieved by the use of few words, if they are rightly chosen, and that attractive presentation is attained rather by clear thinking than by superficial display.

The present series of monographs has been designed with these objects and ideals in view. The task which the authors have been set is no easy one; so that should performance occasionally fall short of intention, the critical reader is asked to echo the words of Goethe that "higher aims, even if unfulfilled, are in themselves more valuable than lower aims quite attained."

E. HOWARD TRIPP.

PREFACE.

SLAVERY to fashion is so engrained in the human race that even the chemist may be forgiven if sometimes he succumbs to it. A recent advance in science may capture the imagination of research workers and open up vistas of unexplored lands awaiting exploitation. Sometimes the soil is fertile and sometimes it is barren, but as a rule it requires to be fertilised by a systematic and detailed study of its peculiarities before it can be turned to profitable account.

This, broadly speaking, has been the history of the use of fluorescence methods for analytical purposes, of which we are at present enjoying (or perhaps suffering from) a vogue. In the early days the results were so encouraging that it was hailed as a rapid, accurate and reproducible method, and for many purposes, indispensable to the analyst. Maturer consideration showed that the accuracy is limited, and the reproducibility is dependent on strict standardisation of working conditions, so that the point has now been reached when it is appropriate to take stock of existing material and sort the wheat from the chaff. The conclusion to be drawn from such a survey is, that if applied with discretion and under standard conditions, fluorescence analysis is a most valuable aid to the scientific worker, especially in routine work or sorting tests, and may usually supplement, though seldom completely replace, ordinary testing or analytical methods.

In our opinion, therefore, there is a real and almost an

urgent need for an up-to-date book which will guide the practical worker through the labyrinth of scientific papers on the subject, many of which are contradictory or too vague to be of real use. This need has been amply confirmed by enquiries among chemists interested in the method, of whom many are unable to test it owing to lack of information, and by the fact that, so far as we know, there is no other such book in the English language.

We have, therefore, endeavoured to make the book appeal to as wide a public as possible by treating (in Part I.) the theory and technique in as simple and brief a manner as possible, due regard being paid to the pitfalls and sources of error which may mislead the beginner.

The second part of the book is more specialised and deals with the applications of the method to a large and varied number of ramifications of pure and applied science. So far as we know we have dealt with all such applications of any real importance, and it will be seen that they are by no means confined to chemistry.

In compiling this second section of the book some difficulty was experienced in selecting material. Where possible the conclusions of the authors of the papers discussed have been checked experimentally, or a definite opinion has been given as to the value of their work. In many cases, however, and particularly where expert knowledge and special materials are required, this has not been practicable, and we have had to be content to give authors' views at their face value. The few cases where this has involved contradictory statements by different workers are due no doubt to different working conditions. The reader seeking to apply the method to his own problems should, however, find these alternatives useful as a guide, and will easily be able to verify one or the other of them by his own technique. An important feature of this section which should appeal to the research worker, is an almost complete classified list of references to papers on the subject, comprising nearly 800 in all.

In conclusion we wish to express our best thanks to the firms who have provided us with information and illustrations of their products (and especially to the Hanovia Quartz Lamp Co. for the loan of a large number of German papers); to Lt.-Col. W. R. Mansfield, M.I.M.E., the London handwriting expert, who has used his new method of luminescence photography for the production of the Luminograms shown at the end of text; and to Mrs. Grant for her continued valuable assistance.

J. A. R.
J. G.

May, 1933.

PREFACE TO THE SECOND EDITION.

WE feel that the fact that we are called upon to prepare a new edition of this work so soon after the date of its first appearance is an ample justification of our view that the subject of Fluorescence Analysis is becoming one of considerable and increasing importance. This is further borne out by the fact that during this period some 300 new papers on the subject have appeared. Many of these deal with entirely new applications of the method; thus, fluorescence microscopy, which has become a science of its own with many ramifications, the use of ultra-violet light for sensitising chemical reactions, and fluorescent indicators may be cited as examples.

We have therefore taken the opportunity of treating such advances fully, as well as revising and bringing the other parts of the book up to date. Certain sections, *e.g.*, those dealing with Paper and Cellulose, Fluorescence Microscopy, Photography, etc., have been completely rewritten, and a wider range of illustrations and Luminograms has been included. Our own added practical experience has also enabled us to speak with more authority on certain of the work of a "doubtful" character which was previously given at its face value. What remains of this has been deleted or only mentioned in passing, but the book has nevertheless increased considerably in size, and the references now number over 1500.

Our special thanks are due to Dr. C. Ainsworth Mitchell, F.I.C., and to Mr. F. L. Garner who have read through Chapters X. and XVII., respectively, and have given valuable advice concerning them; to the individuals and firms who have provided us with further illustrations; and to our numerous friends (including reviewers) who have made suggestions by letter and in print towards the improvement of this new edition.

J. A. R.
J. G.

August, 1935.

PREFACE TO THE THIRD EDITION.

We feel considerable gratification in being called upon to start work on a Third Edition of this book within about two years of the publication of the Second Edition. This confirms our conclusions regarding the continued interest in the subject of fluorescence analysis.

We have once again taken the opportunity of bringing the book up to date in all sections, even at the risk of increasing it again in size. This is inevitable owing to the rate of development of the subject, as is demonstrated by the fact that in the short interval concerned we have filed details of some 800 original papers. We have, however, abandoned any thought of making reference to all of these, but have confined detailed discussion to those which appear to us to be the most important and useful, particularly from the analytical point. By introducing what we have termed "Additional References" after the Bibliographies at the end of each Chapter, we have, however, been able to include references to all papers relevant to the subject, together with a few words indicating the subject-matter in each case. We therefore believe that every important paper on the subject is dealt with in our book, in one way or another. In addition, the Chapter on Textiles and the section on Dyestuffs have been completely rewritten, and the latter now appears as a separate Chapter. The recent important additions to the range of ultra-violet lamps used for this work are dealt with fully, and a number of new illustrations are also included.

In accordance with our past policy we have not hesitated to consult workers on specialised branches of the subject, in order to obtain authoritative opinions on uncertain points and the best material for illustrations. To these helpers, as well as to the numerous other friends who have suggested improvements, we offer our best thanks; a word of special thanks is due to Mr. H. Procter-Smith for his kindness in lending us a large number of scientific journals.

J. A. R.
J. G.

July, 1939.

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PART I.

THEORY AND TECHNIQUE OF FLUORESCENCE ANALYSIS.

CHAPTER I.

THEORETICAL INTRODUCTION.

THE purpose of this chapter is to give the simplest and briefest account of the theoretical side of the subject consistent with the needs of the average worker, who, it is assumed, will be familiar with the more important theories of modern physics.

The Nature of Light.—Physicists of to-day are by no means in complete agreement as to the nature of light, and indeed, there is little immediate prospect of such an agreement being reached. It is, nevertheless, about 300 years since the Dutch physicist, Huyghens, proposed the wave theory, based on an analogy with the transmission of motion along the surface of still water. This was opposed later by the theory that light exerted its effect through the motion of corpuscles, which has in turn been modified or replaced by explanations such as those of Maxwell and Hertz based on electromagnetics.

It is not within our scope to deal with these and the more complex theories current at the present time, and it is only necessary to point out that the one conclusion common to all is that light is a form of energy, and that it is probably electromagnetic in nature.

The medium through which such energy is transmitted is again still the subject of discussion. The conception of the æther, an all-pervading imponderable and incompressible medium, still remains as a working hypothesis. It has been rejected by a few (without however being satisfactorily replaced), accepted by others, and twisted almost out of recognition by some in attempts to make its properties fit their theories. Here, again, we cannot follow the arguments which have led to these conclusions ; they involve considerations ranging from those of the structure of the

atom to that of the universe, and in some cases have hardly emerged from the sphere of speculative philosophy.

For our present purpose, therefore, we must be content to accept a modified wave theory of light, and the existence of an æther as the medium by which the waves are carried. Wave motion of any kind, in its simplest form, is defined by three quantities: (*a*) the *velocity* of travel (V); (*b*) the *wave-length* (λ), which is the distance between two crests (or two troughs) of a wave; (*c*) the *frequency* (n), or the number of vibrations of the wave per second. A moment's consideration will show that these are related by the simple formula,

$$V = n\lambda.$$

In the case of light, the average value of V, for a vacuum, is about 186,000 miles per second and is always constant; sound on the other hand, travels through the air, and only at about 1100 feet per second.

Although the velocity of light is a constant, the wave-length of the constituent rays may vary considerably. The term "light" in fact must be regarded in a very broad sense as including not merely what is apparent to our eyes, but all energy radiated in the form of electromagnetic vibrations. Without complicating matters unnecessarily it may be then said that such vibrations are usually defined by their wave-lengths.

Fig. 1 shows, on a comparative scale, the whole of the known range of electromagnetic vibrations and the means by which they are produced and detected. The wave-lengths are in cms. and Ångstrom units (1 Ångstrom unit, or 1 Å., $= 10^{-8}$ cm.), and the corresponding approximate frequencies are also shown. It will be seen that the radiations may be classified conveniently as follows:—

(1) *The Region of Molecular and Atomic Dimensions, i.e.*, below 140 Å. This class includes the cosmic rays (λ, 0·0005 Å.), the origin of which is still a matter of speculation; γ-rays from radioactive sources (about 1 to 0·005 Å.); X-rays (0·1 to 1000 Å.); and a so-called "intermediate X-ray region" (Holweck, 1922) covering about 10 to 140 Å.

(2) *The Ultra-Violet Region*, with which we are mainly concerned. This is divided into the "near" and "far" ultra-violet and extends from about 136 to 4000 Å.; the rays of the

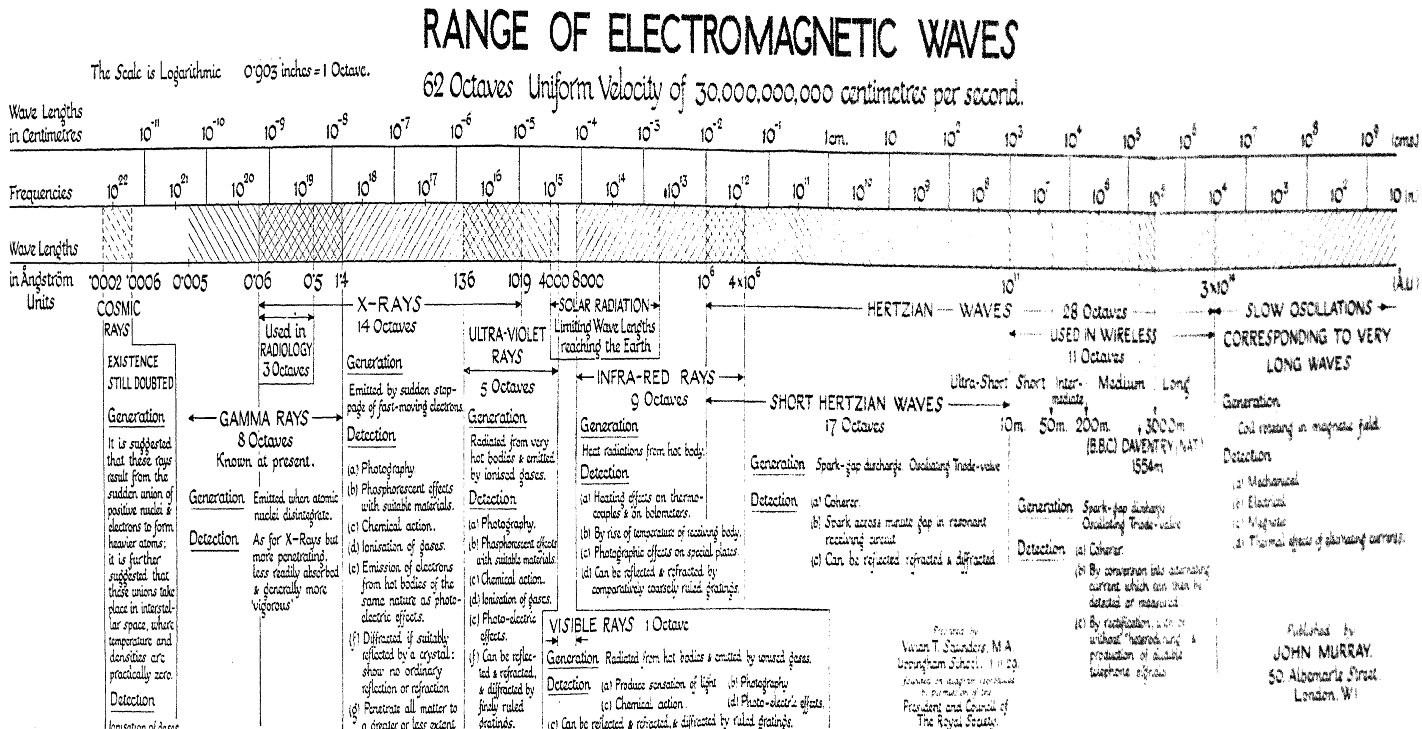

FIG. 1.

(To face page 2.

near ultra-violet have the longest wave-lengths and are neighbours of the violet rays of

(3) *The Solar and Visible Regions*, the extreme wave-length limits of which are from about 0·0003 mm. to 0·03 mm., and from 0·0004 mm. to 0·0008 mm., respectively. The latter region constitutes what we call "white light," which, of course, is the resultant effect on the eye of the colours of the visible spectrum, violet, indigo, blue, green, yellow, orange and red (in order of increasing wave-length). It will be noted that this region, to which the human eye is sensitive, is relatively small compared with the whole range of electromagnetic vibrations and even with the solar region.

(4) *The Infra-Red (and Heat-Ray) Region* overlaps the long wave-length end of the visible region, and so occupies a position analogous to that of the ultra-violet rays at the other end, though it extends to about 0·05 cm., and the overlap is greater. It also overlaps to some extent at the other end of its range (in the heat-ray region) with

(5) *Hertzian Region* (0·01 cm. to 50,000 m.). As a matter of fact, the region to which the name of Hertz (1886) was originally given is between about 1 and 0·01 m., but with the use of rays of such long wave-length for wireless purposes the name has been applied to all such radiations. Wireless waves range in wave-length from about 1 to 50,000 m., and are themselves subdivided into the short (waves below 100 m.), medium, and long (above 1000 m.) regions.

(6) *Region of Slow Oscillations* of wave-length about 10,000,000 m.; this is the extreme known limit of the range.

It will be seen that the subdivisions of this range, which extends from 0·0001 to over 10^{17} Å., are purely arbitrary, being based on the physical effects they produce, and are by no means sharply defined; the influence of astronomical and atomic physics on the explanation of the nature of light will therefore readily be appreciated.

The Ultra-Violet Region.—The position of this region is interesting. It falls between the shortest rays visible to the human eye and the X-ray region of longest wave-length, about which little is known.

Luminescence, Fluorescence and Phosphorescence.—The ultra-violet rays have, however, another useful property other than mere penetration, and this depends on the power possessed

by certain substances to absorb them. All substances absorb electromagnetic vibrations or light, usually over a characteristic range of wave-lengths, and many emit or re-emit such radiations. This emission phenomenon is known by the general term of *luminescence*. The luminescent light may be that of the visible region, in which case it will be seen by the human eye so long as it is not masked by the exciting light ; or, it may fall in some invisible region, and will therefore require special physical instruments for its detection. Luminescence is shown by solids, liquids or gases and is classified as

(*a*) *Fluorescence*, if it lasts during the period of excitation only ; and

(*b*) *Phosphorescence*,[4] if it persists when the exciting source is removed.

Some confusion is attached to the definition of these three terms. Luminescence and fluorescence are often used interchangeably (particularly in German literature) and phosphorescence was originally applied to the emission of light by chemical action, of which the glow accompanying the oxidation of phosphorus (*chemi-luminescence*) is the best-known example. An interesting and more recent example is the intense red glow, observed by J. H. Hellberger,[1] when small quantities at a time of the magnesium salt of phthalocyanine are added to boiling tetralin. Since, in time, this is no longer produced, although it may be restored by addition of a peroxide, it seems that it results from peroxides in the tetralin. Further confusion may arise from the fact that phosphorescence, as defined above, may persist from a few seconds to some months after removal of the exciting source, and the former type may therefore easily be confused with fluorescence. Although it is not strictly accurate, therefore, there is a certain amount of justification for the use of all three terms synonymously. Phosphorescence and fluorescence are probably phases of the same phenomenon, though in general the rays concerned in the latter case are of shorter wave-lengths than in the former (see below [5]). Other forms of luminescence [2, 3] (*e.g.*, *thermoluminescence* and *triboluminescence*,[6] which are produced by heat and by impact or friction, respectively) are mentioned elsewhere. Luminescence may also result from cooling,[5] (*cryoluminescence*), crystallisation (*cristalloluminescence*), or electrochemical action (*galvanoluminescence*), but these are rarer in-

stances. *Bioluminescence* is the term applied to luminescence emitted during biological processes; it is, however, usually regarded as a form of chemiluminescence, and associated with the oxidation of luciferin by oxygen in the presence of the enzyme luciferase.[5]

Theory of Light Emission.[7]—No wholly satisfactory theory has been proposed to account for light emission. The quantum theory and Bohr's atomic model do, however, give us a feasible mental picture of the mechanism accompanying it. In brief, the former theory states that energy is absorbed or emitted in "quanta," *i.e.*, in small units, instead of continuously. All quanta are equal in value, so that an amount of energy may be expressed in terms of the number of quanta with which it corresponds. This theory accounts for a number of phenomena which cannot be explained by the emission of energy in a continuous flow, but its greatest triumph is its application to spectroscopy. The absorption and emission of light by bodies may, in fact, be regarded as a quantum phenomenon.

The pioneer work in this sphere is due to Bohr, whose theory of the atom is well known. Bohr attempted to reconcile the earlier static theories of atomic structure with the spectroscopic properties of the atom, by postulating a number of orbits or shells surrounding the positively-charged nucleus. These orbits are supposed to be occupied by electrons, each of which carries a unit negative electric charge, and each electron, normally, is constrained to move in its particular orbit only. The passage of an electron from one orbit to another, or out of the atom, corresponds with the emission or absorption of energy, and in common with all energy changes, this takes place in quanta. Mathematical deductions along these lines show that a change of an electron from one orbit to another corresponds with an energy-change of one quantum.

The theory has received ample confirmation in many directions, and particularly in its application to spectroscopic phenomena. Nevertheless, in common with most theories, it has its shortcomings, and has therefore been modified so as to eliminate them. The motion of the electrons in their orbits and their rotation about their own axes (analogously with the members of the solar system) are at present still under discussion, but the matter hardly concerns us here.

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If we consider ultra-violet or other radiation to be due to a disturbance in the internal arrangement of the atom, such as the passage of an electron from one orbit to another, it is fairly easy to understand the phenomena on which the method of analysis described in this book, is based. Many substances when exposed to the ultra-violet region of the spectrum appear to absorb energy in this form. Electrons are therefore displaced from their orbits during this process (excitation) and probably pass to orbits farther removed from the nucleus.

An unstable condition is thus set up, and if the electron returns to its original position, there is an emission of radiation. Such changes may also be brought about, in a similar way, by complete loss of an electron from an outer orbit of the atom, and by supplying an electron from an external source to a vacant orbital space.

It has been found, furthermore, that what have been termed "forbidden bands" separating their stable and unstable positions, limit the activities of these electrons.[5] Thus narrow bands may be "jumped," but wide bands form an impassable barrier, and this conception may be used to explain why some substances fluoresce whilst others do not. Similarly, addition of another substance (*e.g.* an impurity) may also help to bridge the forbidden gap and so give rise to the transfer of an electron and therefore, to a fluorescence effect.

Recent work [5] suggests that the term fluorescence should be confined to describe those phenomena which arise as the result of the return of an electron from the state which results from the excitement of energy in the parent atoms. Correspondingly, the term phosphorescence would then be reserved for instances where luminescence is dependent on temperature, the electrons being in the metastable state or completely removed from the parent atoms. Until, however, further data are available concerning these processes, the older definitions given on p. 4 are likely to be preferred.[7]

The light emitted as the result of such a process is not necessarily of the same wave-length as that of the exciting light. Nor is its wave-length always such as to make it visible to the eye, and in addition, unless the substance is phosphorescent as distinct from fluorescent, the bright exciting source may mask its effect. Herein are both the virtue and defect of the use of ultra-violet

rays for the purpose of excitation. Such rays are invisible to the human eye (if "purified" by filtration), so that any reflection of them by the irradiated substance produces no visible interference. On the other hand, the applications of luminescence methods are thereby greatly limited, since they are confined to those substances which emit a radiation visible to the human eye when irradiated with rays of the relatively narrow and invisible ultra-violet region. Incidentally, the fact that it is possible to see the luminescence but not the exciting light, is a special example of the general principle that energy due to radiation tends to be converted into radiation of longer, rather than shorter wave-length.

As a matter of fact, other rays invisible to the human eye will provoke luminescence, X-rays being an example. These, however, are not usually so conveniently generated as ultra-violet light, and so long as care is taken to exclude all visible light, the latter (see p. 10) constitutes the most efficient and easily produced source of excitation. The development of the mercury vapour lamp for therapeutic purposes (sun-ray treatment) has materially assisted this end.

Outside Influences.—Other explanations of luminescence such as surface reflection, have been advanced but are less satisfactory. This applies also to explanations of the far-reaching effects of minute traces of impurities on the luminescence. Pure zinc sulphide, for example, does not luminesce, but the presence of "activators" (see p. 54) such as traces of radium, copper, silver or lead (*e.g.*, in the natural mineral) causes it to do so. On the other hand, certain substances act as inhibitors. Similarly, heat may produce lattice distortion and therefore fluorescence, and in the case of pure zinc sulphide this occurs after 30 mins. at 700° C.

It is also interesting that, in the case of luminescent solutions, there is a concentration for which the effect is greatest, and that both the magnitude of the effect and the wave-length of the light which stimulates it vary according to the solvent used. This probably indicates an explanation based on association between solute and solvent, or perhaps, on ionisation. The effects of other outside influences are discussed in Chapter V.

Fluorescence Analysis.—Where the luminescence produced is characteristic of the substance irradiated, it may be used

as a means of analysis. For ordinary work it is sufficient in most cases to note the intensity and colour, but the method is more specific and is applicable to a greater range of materials if the spectrum of the light emitted is examined spectroscopically. In general, the intensity is proportional to the amount of active substance present, and quantitative methods based on these lines have been described. Luminescence, however, is obtainable only from a restricted number of substances, and such methods are further limited by the fact that it is usually produced only by light whose wave-length falls within a particular range of the ultra-violet region. The usual exciting sources are, however, sufficiently rich in rays of all such wave-lengths.

Methods.—These may be classified as follows :—

(1) *Qualitative.*

(*a*) Direct Irradiation. The substance is placed in ultra-violet light, and the nature, colour and intensity of the luminescence are noted and are compared with the characteristics of that from a genuine sample of known origin. This may be carried out in acid, alkaline or neutral solution, at various concentrations, and in a number of solvents.

(*b*) A refinement is to observe the luminescence under a luminescence-microscope (p. 78), or to determine its spectral characteristics.

(*c*) Chemical Reactions. The suspected substance is treated with a chemical reagent which should produce a luminescent compound, and the appearance of such a compound observed. Conversely, if the substance is itself luminescent, a reagent may be chosen which destroys the luminescence of the substance concerned.

(*d*) Capillary Analysis. This is treated fully on page 59. It is based on the nature of the luminescent zones produced on a strip of filter-paper after it has been soaked in a solution of the sample and dried.

(2) *Quantitative.*

(*a*) Trial and Error. A number of mixtures containing known quantities of the luminescent substance are compared with the sample in ultra-violet light, and an approximate match is obtained with one of them.

(*b*) Photometry. The intensity of the luminescence, other conditions being equal and over a restricted range of concentrations, is proportional to the amount of luminescent substance present, and may be determined photometrically.

(*c*) Capillary analysis (*supra*) lends itself to semi-quantitative work if the intensity of the luminescence of the successive zones is measured photometrically.

(*d*) Fluorescent Indicators (p. 310). Determinations of *p*H value may be made by means of indicators whose luminescence in ultra-violet light changes with change in hydrogen-ion concentration. These, and similar compounds, may be used to indicate the end-points of (*e.g.*) neutralisation or oxidation-reduction titrations, and are usually sensitive in very small quantities and in very great dilutions of reagent, as well as being free from many of the usual errors inherent in the use of indicators.

A change in the luminescence of a particular substance on addition of a reagent may also be used to determine the substance itself. For example, quinine may be titrated in ultra-violet light without the addition of an indicator, the change in luminescence of the quinine being taken as an indication of the end-point (see p. 313).

Finally, it should be pointed out that the application of ultra-violet light to analysis is still in its infancy. The phenomena recorded in the literature are full of stimulating suggestions, some of which have been tested during the compilation of this volume, while the increasing number and variety of published papers on the subject (of which over 2300 are mentioned in this volume) is a proof that the subject still offers ample scope for investigation.

REFERENCES.

[1] J. H. Hellberger, *Naturwiss.*, 1938, **26**, 316.

[2] Soc. Polonaise de Phys., *Rapports sur la Photoluminescence. Acta Phys. Polonica*, 1936, **5**, pp. 431.

[3] T. Muto, *Sci. Papers Inst. Phys. Chem. Res. Tokyo*, 1936, **28**, 207.

[4] H. Rupp, "Die Leuchtmassen und ihre Verwendung," 1938.

[5] Faraday Society. Discussion on "Luminescence," Sept., 1938; *Nature*, 1938, **142**, 779.

[6] F. G. Wick, *ibid.*, 1937, **139**, 603; *J. Opt. Soc. Amer.*, 1937, **27**, 275.

[7] E. Hirschlaff, "Fluorescence and Phosphorescence" (London, Methuen), 1938.

CHAPTER II.

THE PRODUCTION OF ULTRA-VIOLET LIGHT.

IN fluorescence analysis there are several considerations which influence the choice of the source of ultra-violet light.[45] The ideal source should be capable of giving conveniently a fairly intense emission of ultra-violet light of all wave-lengths, and this intensity should not vary greatly with time or with changes in working conditions. Furthermore, it should be steady in its output, free from objectionable fumes or heating effects, and it should be capable of being enclosed and so protected. At present there is no lamp which satisfies all these requirements, although some very nearly do so. It is not proposed to deal here with the sun as a source of ultra-violet light as the subject has been fully treated, for example, by Luckiesh,[1] and is mainly of therapeutic interest. A brief consideration of the artificial sources available in the laboratory is, however, essential.

Lamps.—One of the earliest means of producing ultra-violet light in the laboratory was by a *gas-lamp* burning a mixture of gases or a mixture of a vapour and a gas. In 1902 Wulf[2] designed a lamp in which carbon disulphide vapour was burnt in oxygen, and in 1910, E. Tassilly and R. Cambier[3] substituted nitric oxide for the oxygen in this burner. A flame 20 to 30 cms. high gives an intense white light, and by means of a slit placed opposite the correct region of the flame, a very steady source of light is obtained, which is rich in ultra-violet rays. These and similar vapour lamps are not easy to manipulate and their applications are restricted.

The *high-tension disruptive spark-discharge* between metallic electrodes such as iron, nickel, cobalt, aluminium, tungsten, zinc, magnesium and cadmium provides a source rich in rays of the shorter wave-lengths. With iron electrodes the light emitted is particularly rich in ultra-violet radiation, and the spectral lines are very uniformly distributed. Radiations of

much greater intensities but confined to certain regions of the spectrum, are obtained by the use of electrodes of zinc, magnesium, nickel or cobalt. Ross [4] has found that aluminium electrodes give ultra-violet light, of intensity double that emitted by any of the common metals, but the difficulty with these devices is the maintenance of a constant output. Kowalski [5] found that the oscillating spark between invar electrodes is an economical source of such rays, and F. W. Müller-Essen [6, 7] suggests special iron and tungsten electrodes containing carbon.

K. Götze,[8] in his description of this lamp, points out that the radiant energy falling on a surface is directly proportional to the cosine of the angle of incidence. Müller-Essen uses an *impregnated carbon electrode*, and his paper shows photographs which demonstrate clearly that the resulting spectrum contains many more, and more intense lines than that obtained from a mercury arc (*infra*). Another electrode of this type is of carbon with a core containing 20 parts of nickel, 23 of iron, 4 of aluminium, carbon, and a binder such as potassium silicate.[51]

Another source of ultra-violet light is obtained by striking an *arc between solid electrodes* such as carbon or iron. The arc may be struck in air, *in vacuo*, in hydrogen,[54] or, as is the case in many lamps now available, in a metallic vapour such as that of bismuth, mercury, cadmium or antimony. Iron, carbon and tungsten electrodes are widely employed, the last giving many lines between 2000 and 4000 Å., with a steady output in certain gases such as argon. The disadvantages of this type of lamp for many kinds of work are the evolution of heat and obnoxious fumes, and the disintegration of small pieces of hot metal from the electrodes which causes darkening of the filter.

Table 1, selected from data provided by Haitinger,[9] serves to compare the properties of the various metals suggested for the production of ultra-violet light; and the work of P. Dubouloz [50] may be consulted for the intensity wave-length curves of the carbon arc and the tungsten filament.

A novel source of ultra-violet radiation is a *thorium lamp* described by W: H. Cohn [10] for work in the blue and near ultra-violet spectral regions. A thorium disc ($\frac{9}{16}$-inch diameter, $\frac{1}{16}$-inch thick) mounted on a copper rod, is bombarded by cathode rays in a high vacuum or gas-filled tube; a concave aluminium reflector acts as cathode, while a quartz window

TABLE 1.

Metal.	Intensity Between 2000-8000 Å. (Rowland Units).	3000-4000 Å. (Rowland Units).	Per Cent. of Total Intensity for 3000-4000 Å.	Number of Lines Between 2000-4000 Å.	3000-4000 Å.
Al	2730	2611	95	28	8
Cd	1431	882	68	38	11
Cu	3888	2700	69	1368	187
Fe	7554	3001	40	2390	868
Hg	2905	875	31	78	25
Ni	3036	1238	40	976	309
Pb	3628	2010	55	46	10
W	4804	1405	29	3254	997
Mo	5788	2907	35	3390	1351

opposite the disc allows the passage of the rays. The maximum (D.C.) voltage between the anode and cathode is 2500 volts, and a blue glow then appears corresponding with the passage of a current of 1 milliampere. The range of the spectrum is 2200 to 6000 Å. (maximum 4500 Å.), and this source is preferable to a hydrogen discharge-tube since no lines or bands are present in the longer wave-lengths; it is also superior to the tungsten lamp in that the whole absorption-spectrum of a glass containing ferrous or ferric oxide can be received on one plate, the energy being evenly distributed throughout the entire spectrum. A further advantage is the low working temperature.

The Mercury-Vapour Lamp.—The quartz mercury-vapour lamp,[56, 57] which can be worked with either alternating or direct current and at pressures above or below atmospheric, has found favour in many quarters. According to Buttolph [11] the *spectral-range* extends from 1800 to 14,000 Å., and according to W. T. Anderson [12] even to 40,000 Å., *i.e.*, in the far infra-red region. The radiation of wave-length less than 14,000 Å. is concentrated mainly in a pair of yellow-green lines at 5764 and 5791 and a green line at 5461 Å., and for this reason the visible portion of the spectrum is of unique value as a source of monochromatic light of high intensity for use in polarimetry, spectroscopy and interferometry. The radiation of wave-lengths less than 4500 Å. represents two-thirds of all the wave-lengths less than 14,000 Å., and, similarly to the visible radiation, the principal ultra-violet

lines are concentrated in a few lines of high intensity, *viz.*, two at 3650 to 3654 Å., one at 3984 Å. and possibly another pair between 4046 and 4078 Å.

Dependence on Working Conditions.—The total radiation of the quartz mercury-vapour lamp increases rapidly with increase in pressure in the tube, and depends largely on the temperature of the lamp and the wattage dissipated in a given arc-length. The range of the radiation is also affected by absorption by the luminous mercury vapour in the lamp, and hence the dimensions of the lamp will, to some extent, affect its total output of energy. For a short time after starting the lamp a smell of ozone is noticeable, but when the lamp is running steadily the air ceases to become ozonised, since it appears that the luminous mercury vapour absorbs those rays which produce the ozonisation. The intensity of the radiation increases with the input of electrical energy, that of the ultra-violet and infra-red radiation increasing more rapidly than that of the visible portion of the spectrum. The principal visible and ultra-violet radiations are all emitted by the ionised mercury vapour, and a maximum arc-efficiency is therefore attainable.

Rate of Deterioration.—Coblentz, Long and Kahler [13] showed that the *intensity* of the total radiation decreases to one-half or one-third of its initial value in the course of 1000 to 1500 hours, but that in the first 500 hours there is no appreciable change. W. T. Anderson,[12] however, states that there is a fall in intensity in the first 1 to 2 hours to a constant value, which is maintained for a long time. As most lamps are tested at the works prior to sale, this initial fall has usually occurred before the lamp is packed. The fall is ascribed to physical changes in the quartz which result in it becoming opaque, and also to the quartz becoming attacked and coated with a layer of the reaction-products of the electrodes.[47, 48] This decrease in intensity affects the short and long ultra-violet and the visible portions of the spectrum in nearly equal measure.

After a few thousand hours of operation the light-emission of a quartz mercury-vapour lamp decreases to 70 to 80 per cent. of its initial value, but by adjusting the power input initially so as to obtain a light-emission of about 70 per cent. of the maximum emission, and gradually increasing it as the lamp ages, a constant intensity can be maintained. A practical application of this plan

is described by Anderson,[46] but it appears to be open to the objection that all portions of the spectrum will not respond to the changes in input to the same degree.

Nelson [49] was able to investigate the deterioration of these vapour lamps by the ionising action of the radiation on a charged zinc plate. In his case there was no decrease during the first 500 hours of use, but after 1800 hours the output had fallen by 50 per cent.

Many lamps have an *average life* of about 2500 to 3000 hours and may, even then, usually be cleaned with acid (see p. 17) and re-exhausted, a process which may be carried out about three times before the burner is finally discarded. To minimise the clouding effect the temperature of the lamp should be as low as possible, and some of the lamps on the market, such as the Cooper-Hewitt and Hanovia lamps, have a number of thin vanes near the electrodes which circulate a small current of air and thus exert a cooling action.

Mercury.—According to Moore [14] "chemically pure" mercury is unreliable for ultra-violet lamps. The usual impurities after filtration are copper, lead, iron and bismuth, but over 0·2 mgrm. of total metals per 100 grms. is objectionable. Moore and Grant [15] have both described a simple apparatus for breaking up the mercury and thoroughly washing it twice with fresh 10 per cent. potassium hydroxide solution, twice with 10 per cent. or 1 per cent. nitric acid, and finally with distilled water. After filtration and drying, copper, lead and bismuth should be absent, and the iron (originally present to the extent of about 0·0003 per cent.) should be reduced to about 0·00015 per cent. The process described gives better results than three-fold distillation in a vacuum.

The types of mercury-vapour lamp vary from those giving a point source of light for use in spectroscopy to the large models used for illuminating purposes. Therapeutic models do not, of course, come into consideration here, since their construction is based on entirely different requirements. Some forms of mercury lamp are tubular with small bulbs containing mercury at both ends, into which the electrodes dip. Another design is U-shaped, and by reason of the heating effect, due to the proximity of the two arms, such forms often give a greater yield of ultra-violet light. In such cases it is found that tubes of uniform

bore are unsatisfactory, as the arc tends to take the path nearest the centre of curvature of the bend ; this means that the inner portion of the bend is exposed to a higher temperature, and hence corrosion and porosity develop here more rapidly than elsewhere. This, of course, may be counteracted by making the wall of the tube thicker at these parts. Von Recklinghausen terms this the " Cathode Spot," and proposes to line it with the highly refractive " titanium zircon quartz " and thus to obviate cracking and clouding at this point.

One of the difficulties in working the mercury quartz lamp is that the anode tends to become heated to a higher temperature than the cathode, and the mercury therefore distils from the former to the latter. Kent and Lacell [17] have devised a lamp in an almost circular quartz tube so that the anode and the cathode are thus brought very close together and automatically adjust themselves to the same temperature. Fig. 2 gives some idea of the arrangement of this lamp.

A small side-tube containing magnesium, boron or titanium, is incorporated in some lamps, and when the lamp has been evacuated and sealed, this is heated and the metal then absorbs any residual air ; this device, therefore, also tends to minimise automatically the effects of leaks.

G. S. Forbes and L. J. Heidt [18] improved on the lamps designed by Harrison and Forbes [19] and by Leighton and Forbes.[20] These all operate under constant pressure and give a concentrated discharge ; the leads are not sealed into the lamp and are therefore easily removed, and no special vacuum technique is required. The most recent model has these and extra advantages, since it avoids cemented joints and oscillation of the arc.

The lamp consists essentially of two parallel quartz U-tubes, 30 cms. high and 8 cms. in diameter, joined across one arm. Tapered pyrex plungers ground to fit closely into the walls of the tubes are filled with mercury which serves to make a contact between the wires of the circuit and tungsten electrodes. The arc-space, which connects one arm of each U-tube, is 150 mm. long and may be cleaned or emptied by means of a plugged trap. Water is circulated around the parts which become hot (the mineral salts in tap-water disintegrate quartz in time). The arc is started at 3·5 amps. and 170 volts by heating the mercury,

and a constricted dam connecting the two U-tubes serves to break up the mercury into droplets when it is refluxing, and so returns it to the hot portion of the lamp; this avoids momentary short-circuits.

After 500 hours of use the quartz deteriorates (*cf.* p. 27), but the affected portion may be treated with hydrofluoric acid and

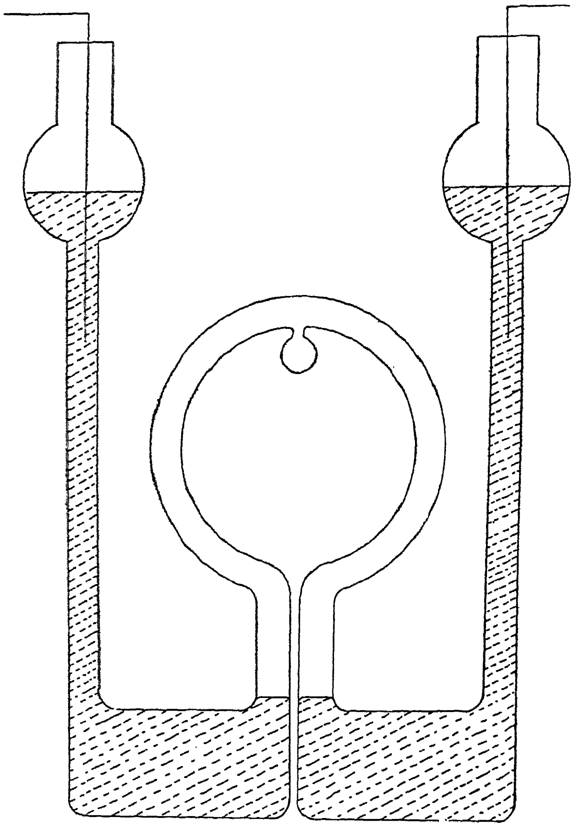


FIG. 2.

heated in an oxygen-gas mixture until it is fairly soft. This may be repeated several times, and the quartz so preserved for 1800 to 2000 hours of use. Total variations in light intensity are seldom more than 3 per cent. A point of interest is the sudden flash which appears when "crystallised" quartz is heated for the purpose of revitrification.

W. Clark [21] gives details for the construction of a similar but smaller mercury-vapour lamp, working at atmospheric pressure, suitable for calibration of spectrometers, preparation of wave-length scales and similar work. It consists of a quartz test-tube containing 1 cm. of clean mercury at the bottom, and a carbon rod reaching to within 1 or 2 mm. of the surface. Contact with the mercury is made by means of a copper wire down the inside of the tube, and the carbon rod is held in position by means of an asbestos-string wad. The arc burns with a current of 0·3 to 3·0 amps., the carbon being made the negative pole in order to avoid a continuous background when the lamp is in use for spectroscopical work. A simple lamp of this type has also been described by T. H. Osgood,[33] and a magnetic device for starting mercury arc lamps is dealt with by L. D. Wilson.[58]

A *bulb-lamp* described by S. English [22] (marketed by the General Electric Co. Ltd.) appears to have certain advantages. It consists of a lamp-bulb made of quartz or glass transparent to ultra-violet light, which fits into an ordinary electric-light holder. Fig. 3 shows the salient points. A are tungsten electrodes, and B, a tungsten filament, the heat from which vaporises the drop of mercury (C), the arc then being struck at A. The radiation obtained requires filtration to remove visible light, and it is also advisable to protect the filter used by interposition of a heat-resisting filter.

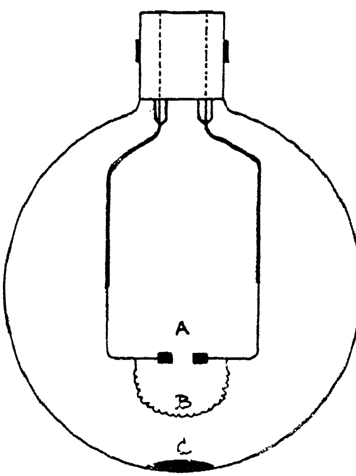


FIG. 3.

An *Hanovia lamp* (Fig. 4A), which is still largely used, consists of the usual evacuated fused quartz tube with a small vessel at either end containing mercury, which is in contact with metallic leads ground into the quartz and fixed by cement. This method facilitates repair and thus gives the burner a longer life. Lamps are made to burn on 110 to 130 volt and 220 volt circuits with either alternating- or direct-current. The burner for alternating-

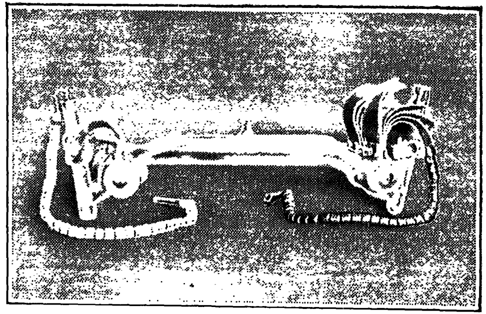
current has two positive poles instead of one as in the case of the direct-current burner, and the fall of potential across the electrodes ranges from 160 to 170 volts.

The suspension of the lamp is arranged so that by means of a handle in the front of the instrument, the burner may be tilted and the mercury made to flow along the floor of the evacuated tube. If the current has previously been switched on, the mercury makes a short-circuit between the two poles; at first the mercury boils and splutters around the negative pole, but as soon as the tube is sufficiently hot and filled with vapour this ceases, and an arc fills the tube. This light is at first bluish and does not reach its maximum intensity before about five minutes.

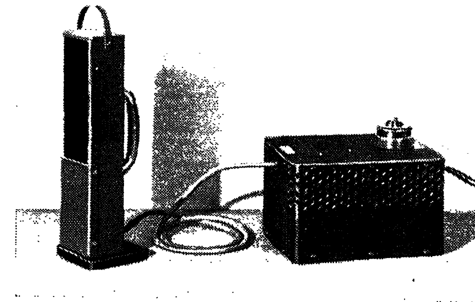
It will be seen from the accompanying illustrations that in the case of the "analytical model" for fluorescence analysis the burner is enclosed, and that the light from the burner passes out through a filter of nickel oxide glass (see Muir,[52] p. 33, and Figs. 4B, 7A and 7B).

In the "S" type of lamp now being developed by Hanovia, Ltd., and described by J. Tutin,[55] the electrodes consist of metal cups coated with electron-emitting salts of the rare earths. Whereas the "K" type burner, having heavy mercury pools for electrodes, necessarily took the form of a horizontal tube with the mercury at the lowest points, the "S" burner can be made in any desired shape, and for operation in any position.

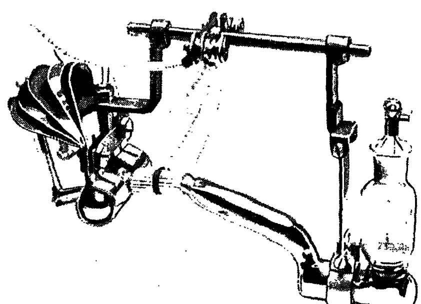
The arc tube has only two electrodes, and will operate on either D.C. or A.C. Argon is introduced as an auxiliary for starting the discharge and no tilting is necessary, a simple switch being all that is required. The mercury content amounts to only a few milligrams, and when thermal equilibrium is reached this all takes the form of super-heated mercury vapour. An "S" burner, unlike the "K" burner, is therefore not subject to variations of internal pressure, within the normal operating range. This leads to important changes in the operating characteristics of the burner. Thus, over the normal operating range the "S" burner voltage varies by only about 2 per cent. for a 10 per cent. change in current, whereas the "K" burner voltage may change by so much as 40 per cent. The presence of only a small quantity of mercury eliminates the possibility of the mercury "hammer" effect when the lamp is being tilted, with the

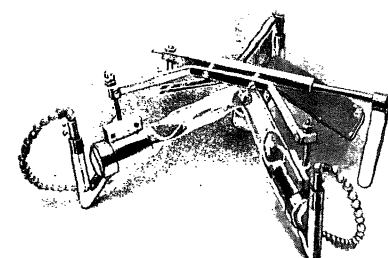


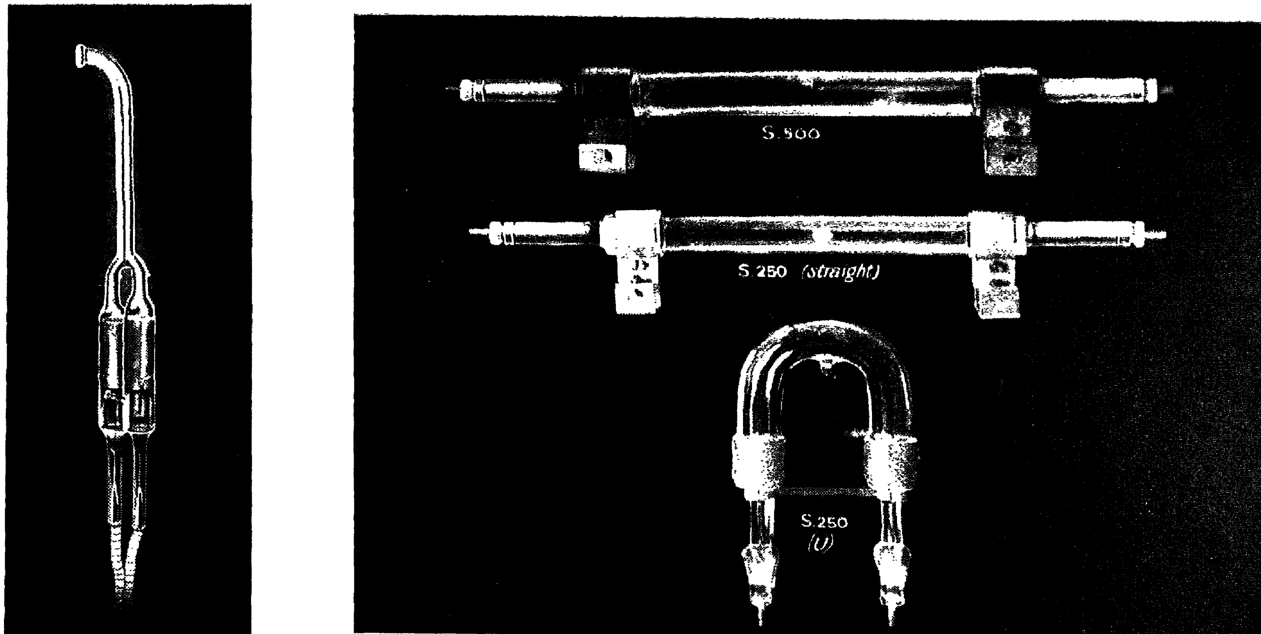
A



B







A B

FIG. 5.

consequent risk of breakage. Examples of burners of this type are shown in Fig. 5B.

Another recent Hanovia model known as the "Universal" type (Fig. 7A) contains an inverted U lamp of the self-starting type, and it can be used on the bench as a suspension lamp, or, by means of a lead, as a hand-lamp (*e.g.*, for the examination of fixed objects, finger-prints, etc.). A simple adjustment enables the instrument to be operated on any D.C. supply, or on A.C. greater than 90 volts.

The "Vi-Tan" lamp, which also was developed (by the Thermal Syndicate Ltd.) for therapeutic work in the first instance, consists of an evacuated U-tube of transparent quartz provided with solid electrodes and containing mercury with neon gas as an activator. Like the other lamps in this class it can be used in any position, and it may be mounted in relatively small vertical cabinets provided with a reflector and a handle in such a way that it can be used conveniently for "close-up" examinations of surfaces, etc. (see Fig. 4B). This and the fact that little heat is evolved (current consumption, approx. 50 watts) are advantages when the object under examination is large or fixed in position. A more compact form of the lamp (Fig. 5A) is used for microscope work, and after passage through an appropriate filter, radiations of wave-lengths between 3000 and 4000 Å. are available for fluorescence tests.

The "Uviation" lamp, made by Kelvin, Bottomley & Baird, Ltd., on the other hand, works at atmospheric pressure, and may also be used in a cabinet. It is operated by means of a series resistance (adjusted for the voltage concerned) from any 5 to 10 amp. D.C. house supply, the connections and lamp being shown in Figs. 6 and 4C. These are self-explanatory, one of the features of the construction of the lamp being the ease with which it can be cleaned. The negative reservoir (N) is provided with cooling fins, and the quartz stopper and wire at the positive end may be drawn out from the diaphragm A in the positive reservoir for transport. The lamp is started by switching on the current, when the heating coil K boils the mercury and the arc strikes in about one minute. A modified design in which a V-shaped burner is used, is now available for microscope work (Fig. 4D); and another recent development is a 3-electrode A.C. burner ("type K") the arrangement of which is such that the burners do not obscure one another.

Since the current through a metal vapour discharge lamp tends to rise continuously while the lamp is in operation, it is usual to compensate for this by means of what is known as a "series ballast." This may take the form of a choke or filament and in one type of lamp described by J. N. Aldington and G. O.

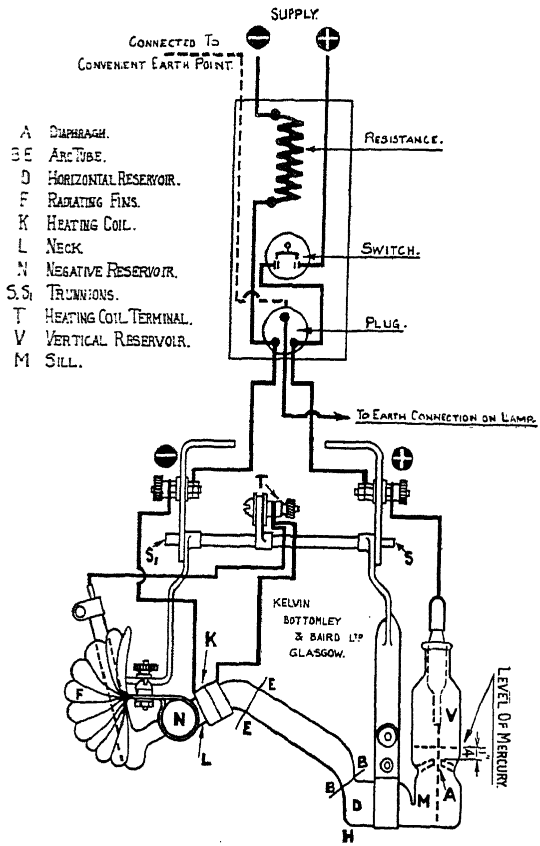
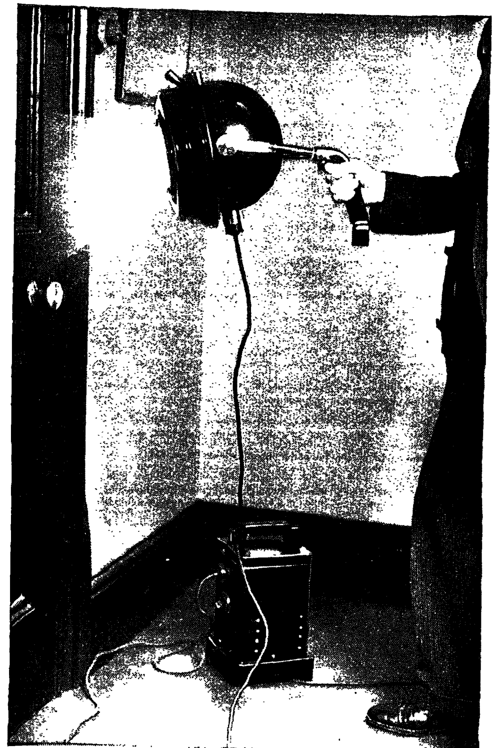


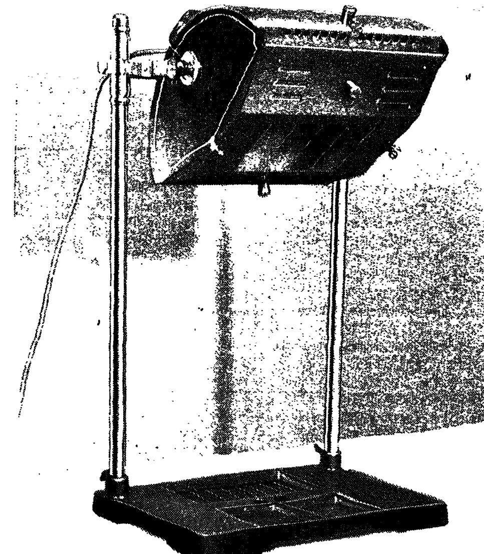
FIG. 6.

[*By courtesy of Messrs. Kelvin, Bottomley & Baird, Ltd.*

Stephens,[59] the latter, when hot, also serves as a source of light. Like many of the modern forms of mercury vapour lamps, this type was designed primarily for the purposes of ordinary illumination (*e.g.*, street lighting), and for this purpose the inside is coated with powders which luminesce and so supply any deficiency in red light.



A



B

FIG. 7.

of Hanovia Ltd

face page 20

The "Mercra" lamp owes its origin to a similar objective, and it is similar in principle. As Fig. 8 shows, it consists of two envelopes, of which the inner is made of transparent quartz and contains the two activated electrodes, whilst the outer envelope is the body of the bulb itself, and for the purpose of fluorescence analysis is made of a filter glass which allows the passage exclusively, or almost so, of ultra-violet light. A third, or auxiliary electrode serves to start the arc automatically as soon as the lamp is connected to the electric mains, the maximum intensity being reached after about 3 minutes; it is stated that 95 per cent. of the resulting radiation is active in inducing fluorescence effects. This lamp can be operated normally only by alternating current, and it should be used in a horizontal position (*i.e.*, at an angle of 90° to the position shown in the illustration), as otherwise the inner envelope throws a shadow on the object under examination. Like most of these lamps, it will not light again once it has been switched off until several minutes have elapsed, and, of course, it cannot be used where unfiltered ultra-violet light is required (*e.g.*, for fading tests, see Chapter XVI), although special lamps with a transparent outer envelope are also obtainable for such purposes.

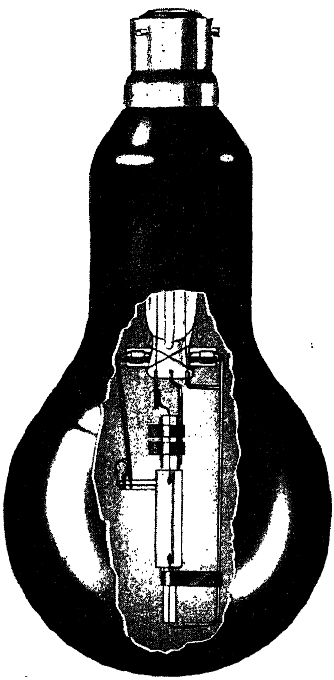


FIG. 8.
[*By courtesy of the British Thomson-Houston Co. Ltd.*

Gallium is now being manufactured by the Vereinigten Chemischen Fabriken[23] of Stassfurt at a comparatively low cost, and has been suggested as a suitable substitute for mercury in arc-lamps, especially when these are used for analytical work. Its particular advantage is that its spectrum contains lines covering a wide frequency-range extending from the ultra-violet to the

middle of the red region. Rare gases are also used in such lamps.[33]

The Callophane.[30]—This is the name given to an instrument (pictured in Figs. 9A and 9B), which has now been placed on the market. It consists essentially of a folding wooden box, 9 × 7 × 1 inches when closed, the lid of which is hinged at one end and fitted with a glass filter transparent only to the ultra-violet rays of daylight. It is necessary, therefore, only to place the object under examination in the box, and to hold the open end against the face as shown, in such a way as to exclude all light, except that which enters through the glass filter. In this way a simple, fairly cheap and portable apparatus is

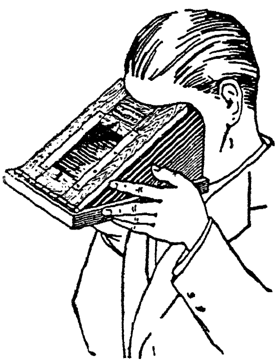


FIG. 9A.

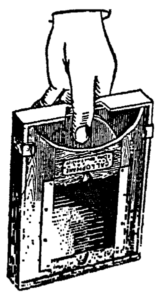


FIG. 9B.

[*By courtesy of Messrs. A. Gallenkamp & Co., Ltd.*

provided for the examination of substances in the ultra-violet light from daylight, which may be used in front of a window or even with an ordinary high-power electric lamp (*e.g.*, 200 watts).

The "Microid" apparatus [35] is an improved form of Callophane, but may be used either with daylight or in conjunction with lamps of the type pictured in Fig. 4 (p. 18).

Needless to say, the strength of the radiation from these "daylight lamps" is considerably less than that from the models already described, but for many purposes they have distinct possibilities.[36-39] When used with a lamp rich in ultra-violet light they are, of course, highly efficient, although the cost plus that of the lamp then gives them little advantage over the usual form of examination cabinet.

Full accounts of these and other less important types of lamps may be obtained from the references given below.[24-44, 53] Special lamps and modified designs of some of the above lamps, which are particularly suitable for photographic work and fluorescence microscopy, are described under these headings in Chapter V.

REFERENCES.

[1] Luckiesh, "Ultra-Violet Radiation" (D. van Nostrand Co., New York), 1922.

[2] Wulf, *Ann. Physik*, 1902, **9**, 946.

[3] E. Tassilly and R. Cambier, *Compt. rend.*, 1910, **151**, 342.

[4] Ross, *Jour. Amer. Chem. Soc.*, 1906, **28**, 786.

[5] Kowalski, *Elect. Rev. West. Elect.*, 1915, **66**, 1055.

[6] F. W. Müller-Essen, *Chem. Ztg.*, 1928, **52**, 37.

[7] F. Müller-Essen, *Chem. Fabrik.*, 1928, 561.

[8] K. Götze, *Chem. Ztg.*, 1929, **53**, 345, 824.

[9] M. Haitinger, *Mikrochemie*, 1931, **9**, 430, 441.

[10] W. H. Cohn, *Jour. Amer. Ceramic Soc.*, 1932, 122.

[11] Buttolph, *Gen. Electr. Rev.*, 1920, **3**, 909.

[12] W. T. Anderson, *The Quartz Lamp*, 1931, **1**, 10; *Radiological Jour. Rad. Soc. of N. America*, 1930, **15**, July.

[13] Coblentz, Long and Kahler, *Jour. Franklin Inst.*, 1919, **187**, 112.

[14] Moore, *Ind. Chem.*, 1932, **8**, 63.

[15] J. Grant, "The Measurement of Hydrogen Ion Concentration" (Longmans, Green & Co., London), 1930.

[16] Anon., "The KBB Mercury Arc" (Kelvin, Bottomley & Baird, London and Glasgow).

[17] Kent and Lacell, B.P. 21,834, 1908.

[18] G. S. Forbes and L. J. Heidt, *Jour. Amer. Chem. Soc.*, 1931, **53**, 4349.

[19] Harrison and Forbes, *Jour. Opt. Soc. Amer.*, 1925, **10**, 6; 1925, **11**, 100; 1926, **12**, 58.

[20] P. A. Leighton and G. S. Forbes, *Jour. Amer. Chem. Soc.*, 1929, **51**, 3550.

[21] W. Clark, *Phot. Jour.*, 1926, **66**, 185.

[22] S. English, *Nature*, 1931, **127**, 310; *General Electric Review*, February, 1930.

[23] Anon., *Chem. Ztg.*, 1932, **56**, 10.

[24] Ellis and Wells, "The Chemical Action of Ultra-Violet Rays" (Chemical Catalogue Co., New York).

[25] L. J. Busse, "Ultra-violette Strahlen und ihre Eigenart" (Sollux, Hanau), 1928.

[26] W. D. McGillivray, "Ultra-Violet Rays and their Properties" (Sollux Publishing Co., Slough), 1927.

[27] J. H. Hibben, *Med. Jour. and Record*, April, 1931.

[28] M. Duyster, *Pharm. Tijdschr. Nederl. Indie.*, 1926, **3**, 127.

[29] F. A. Förster, *Zeit. angew. Chem.*, 1927, **40**, 1414; *Apoth. Ztg.*, 1928, **43**, 45.

[30] Callo, E.P. (Prov.) 15,137.

[31] R. Schnurmann, *Chem. Umschau*, 1928, **33**, 28.

[32] E. Wagner, *Mittlg. der staatl. Potikerschule, Jena,* 1927, 27.
[33] T. H. Osgood, *Jour. Sci. Inst.*, 1928, **5**, 329.
[34] P. Harteck, *Zeit. physikal. Chem.*, 1932, B, 16, 77.
[35] Griffin & Tatlock, Ltd., and J. C. O. Turfery, B.P. 397,059, 27/3/33.
[36] J. Plotnikow, *Zentr. Optik. Mech.*, 1932, **53**, 171 ; *Umschau*, 1932, **36**, 111 ; *Krim. Monats.*, Nov. 1931.
[37] M. Spater, *Kunststoffe*, 1932, **22**, 249.
[38] H. J. van Giffen, *Pharm. Weekblad.*, 1932, 69, 704 ; *Rév. Prod. Chim.*, 1932, 35, 199.
[39] E. Stock, *Farb. Ztg.*, 1933, 38, 1558.
[40] Manhattan Electrical Supply Co. Inc., U.S.P. 1,907,294, 2/5/33 (special seal).
[41] O. Darbyshire, *Jour. Sci. Inst.*, 1933, 10, 322 (robust Hydrogen Lamp for 1850 to 3300 Å.).
[42] A. Hollaender and J. F. Stauffer, *Science*, 1933, 78, 62 (mercury lamp).
[43] P. W. Danckwortt, *Chem. Ztg.*, 1933, **51**, 1018 (portable type).
[44] C. C. Pines, *Amer. Jour. Pharm.*, 1933, **105**, 563 (lamp for minerals).
[45] Forbes, *Jour. Phys. Chem.*, 1928, **32**, 482.
[46] W. T. Anderson, *Rev. Sci. Inst.*, 1934, **5**, 127.
[47] Nogier, *Ill. Eng., London,* 1911, **4**, 503.
[48] Le Bon, *Compt. rend.*, 1911, **153**, 49.
[49] Nelson, *Proc. Amer. Soc. Testing Materials,* 1923, **22**, 485.
[50] P. Dubouloz, *Compt. rend.*, 1933, **196**, 1221.
[51] National Carbon Co. Inc., U.S.P. 1,862,300, 7/6/32.
[52] Anon., *Ind. Chem.*, 1934, **10**, 283.
[53] W. E. Forsythe, B. T. Barnes and M. A. Easley, *Jour. Opt. Soc. Amer.*, 1934, 24, 178.
[54] O. S. Duffendack and J. H. Manley, *ibid.*, 1934, **24**, 222.
[55] J. Tutin, *Elec. Rev.*, 1 and 22 Feb., 1935.
[56] B. D. H. Watters, *Brit. J. Med. Res.*, 1936, Jan. (History and development of the quartz mercury arc).
[57] G. Heller, *Philips' Tech. Rev.*, 1936, No. 1 (Theory of the mercury vapour lamp and conditions governing its efficiency, are discussed).
[58] L. D. Wilson, *J. Amer. Chem. Soc.*, 1936, 58, 856.
[59] J. N. Aldington and G. O. Stephens, *Siemen's Mag. Engineering Suppl.*, 1938, May.
[60] D. A. Derrett-Smith, *J. Text. Inst.*, 1937, 28, 119.

ADDITIONAL REFERENCES.

H. P. Hood, U.S.P. 1,964,321, 26/6/34 (Glass jacket for ultra-violet lamps consisting of SiO_2 55-80, B_2O_3 10-25 and Na_2O 10-25).
A. Münzinger, *Nitrocell.*, 1935, 6, 179 (New ultra-violet lamp, covering the infra-red and visible regions also).
N. V. Philips' Gloeilampenfabriken, B.P. 448,685, 6/3/36 (Mercury vapour lamp operated at a pressure exceeding 10 atmospheres, and containing strontium oxide to increase emission).
H. Wrighton, *J. Roy. Microscop. Soc.*, 1937, **57**, 260 (New mercury vapour lamp, particularly suitable for fluorescence microscopy).
A. van Wijk, *Philips' Tech. Rev.*, 1938, Jan. (Description of the mercury vapour lamp and its uses).

CHAPTER III.

FILTERS.

Effects of the Rays on the Eyes.—It has long been known that intense or prolonged radiation, especially by rays of short wave-length, causes painful irritation of the tissues of the eye. This effect is observed in cases of "snow blindness," where the reflection of the solar radiation by snow (which is a very efficient reflector of ultra-violet rays) is the active agent; another instance is "eclipse blindness" caused by the direct action on the eyes of the rays of the sun when it is observed in partial eclipse. The pain has been likened to that of grit in the eye, and may take some time to appear if the intensity of the activating rays is low. The effective radiation generally has a wave-length shorter than that of the rays of the solar spectrum, the middle and extreme ultra-violet regions from artificial sources being especially active.

Verhoeffer and Bell [1] found that radiations of wave-length shorter than 3050 Å. are able to injure the cells of the eye by chemical action, but for wave-lengths longer than this, the effect of the light is successfully opposed by the physiological action of the cells. Ultra-violet light from artificial sources is generally very intense and contains a large percentage of the injurious rays, so that care should be taken not to expose the eyes to the light from such a source for any length of time. Unless the lamp is totally enclosed, goggles should be worn which protect the eye on all sides, as ultra-violet light can be reflected from walls and polished surfaces in the same manner as visible light (see also publications by H. T. Plank [2] and by W. Coblentz and C. W. Hughes [3]). In 1914 Crookes [4] made over 300 glasses of different compositions and investigated their spectral characteristics in order to find a colourless specimen which would absorb ultra-violet and infra-red rays. He did not achieve his object completely, but did, however, discover a glass with a bluish tinge

which absorbed about 90 per cent. of the objectionable radiation.

Glasses which Absorb Ultra-Violet Rays.—Commercial filter-glasses vary greatly in composition, and this variation is often so very indefinite that no useful purpose will be served in presenting other than general data (see Grant [33] and Jentzsch-Graefe [37]). Glasses which do not absorb the ray 3650 Å. should be rejected when a selection is being made for goggles, and this excludes the choice of " pebble " or natural clear quartz for lenses. Fortunately, the most objectionable rays are those shorter than 3400 Å., and these are more or less completely absorbed by the majority of ordinary commercial glasses of moderate thickness (about 2 mm.).

Coblentz and Emerson [5] suggest a number of specially *coloured glasses* for the protection of the eyes, and these include all colours from black to amber, green, greenish-yellow and red. Glasses containing titanium, vanadium and cerium in the oxidised form all absorb ultra-violet light and are either colourless or only slightly coloured. Lead glass, however, reduces the transparency of the glass in the ultra-violet region, whilst cobalt and nickel, both of which give blue glass, and manganese, which gives a purple glass, have little or no effect on the near ultra-violet region. Cobalt glass appears to be even more transparent to rays of a wave-length about 3000 Å. than to clear glass.[6] H. Seabrook,[7] however, has patented a glass which, he claims, is strongly absorbent in the ultra-violet and infra-red regions, and contains 0·3 to 6 per cent. of cobalt and iron oxides on the weight of the finished product.

The transparency of the so-called flint glasses does not usually extend so far into the ultra-violet region as that of the crown glasses; in other words, glasses of high refractive index are more likely to be less transparent in the region of 3100 Å. than those of lower refractive index. Arsenic or antimony oxide added to the mix increases the absorption of heat (*i.e.*, infra-red) rays. About 1 per cent. of chromium in a glass of 1 to 2 mm. thickness absorbs ultra-violet rays almost completely. According to W. E. S. Turner [8] the transmission of glasses containing iron is closely connected with the iron oxide content, since ferrous iron absorbs ultra-violet light to a less extent than iron in the ferric state, and the deterioration of such glasses (see p. 114) may be accounted for by slow oxidation of the iron.

Glasses which Transmit Ultra-Violet Light.—W. E. S. Turner and D. Starkie [9] examined a number of commercial glasses covering the ranges 7000 to 2000 Å. and 2950 to 3150 Å. (which, however, is usually of therapeutic interest), and the following percentage transmissions were observed :—

Corex, 89	Sanalux, 73.	Sun Ray, 62.	Holvi, 61.
Vita, 54.	Helio, 52.	Uviol, 46.	Quartz Lite, 4.

Ordinary window-glass (see also H. Valentin [35]) is taken as unity. After use the various glasses showed a decrease in power of transmission varying from 13 per cent. for Vita glass to 7 per cent. for Sanalux. W. W. Coblentz and R. Stair [64] showed, however, that on exposure an increase or decrease in transmittance can occur according to the wave-lengths of the constituent rays of the light used, and that in some cases (*e.g.*, with sunlight) these can proceed simultaneously and neutralise one another in effect. Goodman and Anderson [38] have examined a number of glasses to determine their transmission characteristics in the region of 2975 Å., which is of interest from the therapeutic point of view, and Table 2 shows their results.

TABLE 2.

Name of Glass.	Thickness, mm.	Qualitative Limits.	Percentage Transmission of Incident Light.
Window glass (unknown origin)	3	3130	0·0
Quartz Lite . . .	2	3130	0·0
Fused quartz . . .	2	2300	85·0
Corex (clear) . . .	4	2300	59·0
Vioray	2·5	2800	38·0
Vita glass (plain) . .	2	2650	27·0
Vita glass (cathedral) . .	3·9	2800	27·0
Cello glass (modern) . .	—	2900	23·0

These results are confirmed by Lunelund [40] who used the region 3200 to 2900 Å.

J. Eisenbrand [10] has examined the percentage of ultra-violet light of various wave-lengths passing through a glass plate 7 mm. thick ; he obtained the following results :—

Wave-length, Å.	*Per cent. passing through*
3660	90
3340	14
3130	under 1

P. Gilard and his co-workers,[11] and also Kögel,[30] Becker [66] and W. Lenz,[31] have investigated the effects of different substances on the transmission of ultra-violet light by various glasses. Transmission increases with increase in silicon content until the silicon reaches an optimum concentration, which varies according to the nature of the other components. The concentration of calcium seems to be immaterial, whilst an increase in barium or zinc content is favourable to greater transmission. Addition of boric acid extends the range of transparency, and potassium is more effective than sodium in increasing the transparency.

The transparency of metaphosphate glasses has been measured by J. A. Benkiser and F. Draisback,[79] who concludes that this extends further into the ultra-violet region the lower the atomic number of the cation present. Glasses formed by fusing the lead, barium, calcium and beryllium salts show transparencies extending to 3250, 3100, 2400 and 2140 Å., respectively. The use of lead metaphosphate in conjunction with calcium, magnesium or sodium salts, or mixtures of these, improves the transparency of the resulting glass produced.

A Corning Glass Works patent [39] for filters which transmit ultra-violet light claims the use of silicon dioxide 0 to 43 parts, alumina 0 to 30 parts, boric anhydride 5 to 70 parts, calcium (with calcium fluoride) 5 to 50 parts, cobalt 5 parts, and ferric oxide or titania, the melt being carried out in a graphite vessel. Fig. 10 shows the spectra obtained with various glasses as filters. K. Fuwa [80] employs as a melt to obtain a glass transmitting ultra-violet light, a mixture of silica 68, boric anhydride 16, sodium (as Na_2O) 15, and potassium fluoride 1 per cent. The latter is added to increase the workability of the melt, and to the fused mass is also added about 0·5 per cent. of potassium tartrate to reduce the ferric iron present to the ferrous state; this further increases the transparency of the glass to ultra-violet light (see p. 113).

It would appear from the work of J. Sugie [12] that an increase in the transparency with diminution of thickness is smaller in the less transparent glasses than in the more transparent kinds.

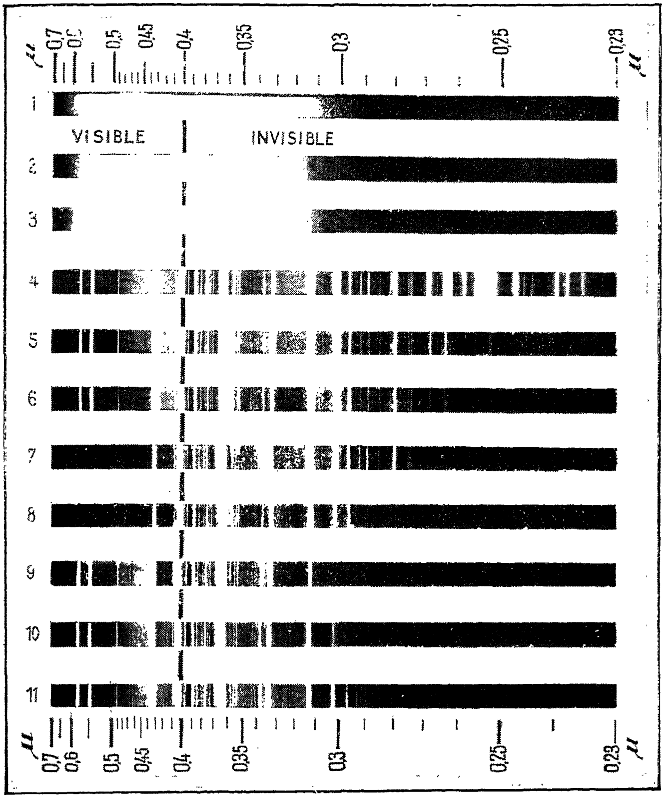


FIG. 10.

1. Sunlight (at sea level).
2. Carbon arc lamp.
3. Sollux lamp (tungsten filament).
4. Mercury quartz lamp (without filter).
5. Mercury quartz lamp through 1·3 mm. white uviol glass.
6. Mercury quartz lamp through 2·6 mm. white uviol glass.
7. Mercury quartz lamp through 1·3 mm. blue uviol glass.
8. Mercury quartz lamp through 2·6 mm. blue uviol glass.
9. Mercury quartz lamp through thin window glass.
10. Mercury quartz lamp through 0·25 mm. celluloid sheet.
11. Mercury quartz lamp through 0·05 mm. mica sheet.

[*By courtesy of The British Hanovia Quartz Lamp Co. Ltd.*

[*To face page* 28.

Fritsch [13] has described a durable glass which can easily be made in the laboratory and which transmits ultra-violet light down to 1850 Å.; 6 grms. of commercial calcium fluoride and 14 grms. of boric acid are powdered, mixed and melted together. The melt is then poured on to platinum foil and allowed to cool slowly. A formaldehyde-urea condensation product (" Aldur ") has a transmission of 42 to 72 per cent. between 2540 and 3600 Å.,[14] and crystal-clear cellulose acetate (" Lumarith ") will transmit 20 per cent. of the ultra-violet rays of the sun through a sheet 0·0035 in. thick.[51] C. Dreyfus [81] has taken advantage of the transparency of cellulose derivatives to use them as the binding medium in laminated glass, the two glass layers used having a high silica content. The transmission of Cellophane has been studied by E. Gilles,[82] who finds that the absorption of ultra-violet light is variable, and considerable at 2200-2600 Å.; vegetable membranes transmit radiations of wave-length 2397 Å.

The vessel containing the substance under examination, of course, should itself be non-fluorescent so that no confusion results, and vessels coated with non-fluorescent animal charcoal, or made from some non-fluorescent glass, should be used. Many glasses fluoresce (*cf.* p. 113), generally a greenish, or a greenish-yellow colour (*e.g.*, Pyrex, Erseco, and some Thüringian glasses), whilst Durax and Supremax have a reddish fluorescence; such glasses are therefore unsuitable for observation tubes or containers for the examination of fluorescent substances. M. Haitinger and his co-workers [15] have examined a number of glasses and find that ordinary thermometer glass, old Jena glass apparatus, Suprax, Dupran and Austrian glass apparatus, and some kinds of Thüringian and Kavalier (Bohemian) glass do not fluoresce.

Filters other than of Glass.—A number of filters made of substances other than glass are used to *absorb* ultra-violet or visible light. As early as 1909 Mannich [41] suggested the use of triphenyl methane, cinchonine sulphate, etc., for absorbing ultra-violet rays, and a patent by Clark [42] claims the use of gelatine impregnated with a salt of glucose phenylosazone *pp'*-dicarboxylic acid. The line 3132 Å. from the mercury-vapour lamp is transmitted by some commercial dark filters, but can be eliminated by the use of a cell 6 cms. thick containing a saturated solution of uric acid. For the ultra-violet absorption the I.G. Farbenind., A.-G.[16] have patented the use of films of cellulose derivatives, or

of the condensation products of urea with formaldehyde, which can be stained with fuchsin, crystal violet or other organic dyes or inorganic pigments, whilst in another patent [17] it is proposed to incorporate with them quinine or æsculin which also absorbs the ultra-violet light. F. Zernik [18] uses hydroxyquinoline sulphonic acids and their salts or esters for colourless filters, and T. Suzuki and S. Sakurai [19] suggest the sodium or potassium salts of the acid obtained by the condensation of dextrose with phenylhydrazine para-sulphonic acid in the presence of sodium or potassium acetate. It is claimed that a layer 2 cms. thick of a 0·01 per cent. solution completely absorbs the ultra-violet but transmits most of the visible rays. G. Heyne and M. Schön [83] find that thin layers of Durophen lacquer 218V is opaque to light of wave-length less than 4000 Å., but are practically non-absorbent for visible light.

F. Schlemmer and F. Maier [68] have examined the catalytic auto-oxidation of butter-fat in ultra-violet light, and find that the use of a wrapping material which excludes light of wave-lengths between 3540-2000 Å. prevents the production of tallowiness but not of bacterial rancidity. With the object of preventing rancidity, a number of substances designed to render wrappings impervious to ultra-violet light have been patented. C. Joseph [69] claims the use of non-glucosidal derivatives of coumarin (*e.g.*, umbelliferone acetic acid ethyl ester), and the use of N-alkyl derivatives of amino-benzophenones are covered by DuPont and Nemours.[70] Pyrene and its colourless derivatives, *e.g.*, pyrene 3 : 5 disulphonic acid, have also been claimed.[71]

Many of the above substances are soluble in water and may therefore, be removed by moisture from the food which condenses on the inside of the exposed wrappers ; hence substances showing affinity for the wrapping material have been proposed (so-called " colourless dyestuffs "). O. Gerngross [72] claims the use of pine or quebracho extract, the tanning agents being washed out of the films or wrappers after the initial treatment (see p. 214). Benz-triazole derivatives derived from *o*-substituted arylamines,[73] and the sodium salt of the urea derived from 4-4′-aminobenzoyl-amino-aniline sulphonic acid [74] are other types of compounds which have been patented for use in this connection.

An ultra-violet light filter for rays having wave-lengths between 2900 and 3200 Å., comprising a film of a compound having the formula, $R(OH)CO_2NH(C_2H_4OH)_3$, where R is a benzene

or naphthalene nucleus having the hydroxyl and carboxyl groups in the *ortho* position in respect to one another, has been claimed by F. E. Stockelbach.[75] Other relevant patents of interest are to be found in the references.[76, 77]

Miethe (*cf.* pp. 69, 284) suggests the use of a solution of cerium ammonium nitrate for photographic reproductions of fluorescent phenomena in order to cut out any reflected ultra-violet light, but a cadmium glass filter may also be used, although an exposure of from four to five times that required in the first case is necessary. The latter glass fluoresces with a ruby-red colour, but this does not matter if plates treated with erythrosin are used. Other solutions for use in fluorescence photography consist of a 2 per cent. solution of sodium nitrite, and solutions of some of the nitrophenols in dilute sulphuric acid (Gaumont [20, 49]); another light filter due to J. Shoji [21] consists of a film containing hexamethylene tetramine triphenol made by condensing phenol with formaldehyde in the presence of ammonia, and J. A. Radley has used a layer of a solution of *o*- or *m*-nitro benzaldehyde 3 mm. thick. Grünsteidl [43] suggests that fluorescent phenomena should be observed through a potassium nitrite solution filter in order to cut out any reflected ultra-violet light which would cause the eye to fluoresce and thus give a fictitious colour value.

It is, however, usually more important to know what glasses or filters will allow *the transmission of ultra-violet rays*, but will absorb the rays of the visible region. Wood [22] used a cell containing a solution of nitroso-dimethylaniline of such a strength as just to eliminate all of the blue and most of the violet regions, since it is fairly transparent to ultra-violet light between 2800 and 4000 Å. In 1914 he used a filter [48] containing methyl violet-4R and nitroso-dimethylaniline which passes the line 3660 Å. and also to a less extent 3984 Å. N. R. Dhar [44] also recommends methyl violet and nitroso-dimethylaniline, with the No. 18 ultra-violet Wratten Filter, and G 586 AW8 10 mm. filter of the Corning Glass Co. for the lines 3650, 3656 and 3663 Å. Lehmann [23] used gelatine impregnated with *p*-nitroso-dimethylaniline, and a second cell containing copper sulphate solution served to absorb the red rays of the spectrum. Many combinations of solutions of aniline dyes or of filters of dyed gelatine may be used for isolating portions of the spectrum of the near ultra-violet region, but in general, it is necessary to rely on gases and a few other media

for the isolation of portions of the middle and extreme ultra-violet regions. Miethe and Stengel [24] have given a list of dyes suitable for such filters, with the ranges of wave-length which are transmitted at given concentrations. L. A. Jones [25] also gives a series of eight filters of good stability suitable for the isolation of spectral bands in the infra-red, visible and ultra-violet regions. They are constructed from a combination of Wratten dyed-gelatine filters, various grades of Corning glass, and from aqueous solutions of nickel and copper sulphates. D. W. Dana [84] recommends the use of Corning Glass No. 038, Nuviol A when the radiation from the source of ultra-violet light extends into the visible violet.

Plotnikow [26] finds that *dyes* in general are often too unstable to ultra-violet light to be of much permanent use, whilst cells filled with solutions are not convenient to handle, and, moreover, the concentration of the constituents must be carefully regulated as too little dye will allow visible light to pass, and too much will seriously diminish the intensity of the ultra-violet light; some dyes are, however, useful in combination with gelatine on a clear glass backing. A combination of aniline-green and resorcin-blue isolates the ray 3660 Å. with little diminution in intensity, but acid-green and methyl violet are better for the transmission of this ray. Such filters are often sensitive to changes in temperature.[36]

L. T. M. Gray [27] has investigated the spectral transmissions of a number of commercial filters, including the Luther and Forbes G. 1. filter [28] and Winther's 3650 Å. filter.[29] The former is made by dissolving 0·0785 grm. of fuchsin-*S*, 0·0164 grm. of methylene blue and 0·0410 grm. of *p*-nitroso-dimethylaniline in 1 litre of water, and Gray finds that 0·78 cm. of this solution in a glass cell transmits rays from 3300 to 3800 Å., with a maximum transmission of only 8 per cent. at 3650 Å. On dilution to one-fifth of this strength, a thickness of 2 cms. transmitted 30 per cent. of the intensity of the ray at 3600 Å. This filter is therefore efficient qualitatively but not quantitatively; it is also very sensitive to changes in *p*H value. Winther's "3650 Å. filter," in a thickness of 1 cm., gave a transmission for this ray of 35 per cent. W. V. Bhagwat [45, 78] and also H. L. J. Bäckström [46] have investigated the use of cobalt and nickel salts in solution for absorbing rays of long wave-lengths and trans-

mitting the ultra-violet. The last-named worker recommends a mixed solution of 1·75 *M* nickel sulphate, and 0·5 *M* cobalt sulphate in layers 3 cms. thick for absorbing the former and passing the line 3660 Å.; the line 3340 Å. is transmitted also, but only weakly, and the percentage transmission of incident light is 72 for the line 3130, and 80 for the line 2600 Å.

For the isolation of the line 3130 Å. an additional filter, 1 cm. thick of 0·025 *M* potassium phthalate, is required for transmitted light consisting of 96, 3·5 and 0·5 per cent. of radiation of wavelength 3130 Å., 3340 Å. and over 3340 Å., respectively; 85 per cent. of the incident radiation of the line 3130 Å., and less than 0·1 per cent. of the line 3020 Å., are transmitted by this filter. The lines 2900 Å. and less may be isolated by cellophane impregnated with sodium benzoate or potassium hydrogen phthalate,[50] and 1 to 5 per cent. each of sodium fluoride and ammonium oxalate has also been used.[56] J. J. Fox has suggested that chlorine and bromine filters should be used to isolate the little-used but very active mercury line 2537 Å.; this should be particularly suitable for microscopic work (p. 78). E. J. Bowen,[63] J. Doetsch[67] and B. K. Vaidya[65] have formulated a comprehensive list of single and composite chemical filters for the isolation of the most important lines between 2480 and 5790 Å.

Lenses of *quartz* coated with silver transmit ultra-violet light but not visible rays (see also p. 79 and below). It is best to deposit the silver on the quartz by reducing an alkaline solution of a silver salt with lactose, and this gives better results than thin silver foil, since it cuts off rays of the extreme (*i.e.* short wavelength) ultra-violet region.

"*Wood's glass*" appears nearly black in ordinary light, as it cuts out practically the whole of the visible spectrum, although it is transparent to most of the ultra-violet region. This glass is used extensively in fluorescence work, and has been incorporated in a number of lamps for use in analytical work. It is essentially a nickel oxide glass, and a glass of this type produced by the Corning Glass Works contains, according to the French patent specification, from 0·5 to 12 per cent. of nickel oxide. The best glass appears to have the composition: silica 50, barium oxide 25, potassium oxide 19, and nickel oxide 9 per cent.; 1 per cent. of copper oxide is added to cut out red rays. It is important to bear in mind that the spectral and

transmission characteristics of such glasses may vary from batch to batch, and even from sheet to sheet.[34] On looking at the sun or, better, at an ultra-violet lamp through a piece of this glass, a dull reddish area on a black background is all that can be seen; this probably is not due to transmitted visible light, but to the fluorescence of the vitreous humour and other eye substances under the action of the transmitted ultra-violet light, but it is difficult to differentiate between this phenomenon and the normal limits of visibility of ultra-violet light, which of course vary with the individual.[55-60] Secondary transmission in the red also, probably accounts for the colour of the image seen.

H. J. Taylor [61] has pointed out that age is an important factor in this connection, since the ray 3130 Å. is visible by individuals between the ages of 15 and 30, whilst the elderly can see only down to 3650 Å. W. de Groot [62] plotted the age of his subjects against the visibility ratios for 3650 and 4947 Å. and for 3130 and 4047 Å., and confirmed the decrease in powers of visibility between the ages of 30 and 45. It is doubtful whether such a method could be used to estimate age, since accidental properties of the eye (*e.g.* colour) may also play an important part in determining the visibility of a particular ray.

P. Carnot and R. Coquoin [54] have described a substitute for Wood's glass made by soaking a sheet of gelatine in a solution containing 1 per cent. of silver nitrate and 10 per cent. of ammonia for 10 minutes, reduced silver being subsequently produced by immersion in an alkaline 10 per cent. solution of formalin (*cf.* Brock, p. 71). L. Bloch [32] and P. W. Danckwortt and E. Jürgens [53] have proposed similar methods of comparing the transparency of glasses towards ultra-violet light; one is worthy of mention. The glass is held between a fluorescent screen, or a sheet of white fluorescent paper, and the source of light; the more ultra-violet light absorbed by the glass, the less fluorescent the paper appears. The fluorescence can then be compared on a graded light-scale or against a wedge of glass of known transparency. A. H. Taylor [52] gives data for the reflecting power of numerous materials (paints, alloys, etc.) towards ultra-violet light (2967 Å.) and daylight. The method has also been used by R. Lassé,[47] who, however, employed a uranium-glass plate instead of paper as the fluorescing screen; in his study of the solarisation of glasses (*i.e.* the change in their transmission

characteristics with exposure and ageing, etc.) he also used the darkening of a photographic plate as a method of estimation, and he considered this to be even better.

REFERENCES.

[1] Verhoeffer and Bell, *Proc. Amer. Acad. Arts and Sci.*, 1916, **51**, 640.

[2] H. T. Plank, "Actinotherapy and Allied Physical Therapy," Chicago, 1926.

[3] W. Coblentz and C. W. Hughes, U.S. Bureau of Standards, *Scientific Paper*, No. 439, 1924.

[4] W. Crookes, *Phil. Trans.*, 1914, (i), 214.

[5] W. Coblentz and Emerson, U.S. Bureau of Standards, *Tech. Paper*, No. 93, 1917.

[6] M. Luckiesh, *Jour. Franklin Inst.*, 1918, 186, 111.

[7] H. Seabrook, B.P. 328,520, 1929.

[8] W. E. S. Turner, *Trans. Soc. Chem. Ind.*, 1929, 48, 65.

[9] W. E. S. Turner and D. Starkie, *J. Soc. Glass Tech.*, 1928, **12**, 306.

[10] J. Eisenbrand, *Pharm. Ztg.*, 1929, **74**, 263.

[11] P. Gilard, P. Swings and A. Hautot, *Bull. Acad. Roy. Belg.*, 1931, **17**, 235.

[12] J. Sugie, *Osaka Ind. Lab. Bull.*, 1924, **5**, 26.

[13] Fritsch, *Phys. Zeit.*, 1907, **8**, 518.

[14] R. H. Crist, *Jour. Ind. and Eng. Chem.*, 1928, **20**, 1367.

[15] M. Haitinger, H. Jörg and V. Reich, *Zeit. angew. Chem.*, 1928, **41**, 815.

[16] I.G. Farbenind., A.-G., B.P. 322,200, 1928; 424,366, 2/12/33.

[17] I.G. Farbenind., A.-G., G.P. 499,017, 1929.

[18] F. Zernik, U.S.P. 1,688,259, 1928.

[19] T. Suzuki and S. Sakurai, E.P. 242,721, 1924.

[20] Gaumont and Soc. des Établissements, Fr. P. 686,905, 1929.

[21] T. Shoji, U.S.P. 1,751,220.

[22] Wood, *Phil. Mag.*, 1903, **5**, 257.

[23] H. Lehmann, *Phys. Zeit.*, 1910, **11**, 1039.

[24] Miethe and Stengel, *Zeit. wiss. Phot.*, 1919, **19**, 57.

[25] L. A. Jones, *Phot. Jour.*, 1930, **70**, 337.

[26] Plotnikow, "Photochemiker Versuchstecknik," Leipzig, 1912.

[27] L. T. M. Gray, *Jour. Phys. Chem.*, 1927, **51**, 1732.

[28] Luther and Forbes, *J. Amer. Chem. Soc.*, 1909, **31**, 770.

[29] Winther, *Zeit. Elektrochem.*, 1913, **19**, 389.

[30] Kögel, *Chem. Fab.*, 1928, **1**, 55.

[31] W. Lenz, *Zeit. anal. Chem.*, 1915, **54**, 27.

[32] L. Bloch, *Deut. Opt. Woch.*, 1927, Nos. 40 and 41.

[33] J. Grant, *Glass*, 1932, **9**, 391.

[34] Anderson, Private Communication.

[35] H. Valentin, *Pharm. Zentr.*, 1933, **74**, 5.

[36] H. Kohn and K. von Fragstein, *Physikal. Zeit.*, 1932, **33**, 929.

[37] Jentzsch-Graefe, *Zeit. Phys. Chem. Unterricht.*, 1919, **32**, 181.

[38] H. Goodman and W. T. Anderson, *Amer. Jour. Phys. Therapy*, 1928, **5**, 17.

[39] Corning Glass Works, U.S.P. 1,886,280, 1/11/32.

[40] H. Lunelund, *Tekn. Aikakaus.*, 1932, **22**, 414; through *Brit. Chem. Abst.*, B., 1933, 465.

[41] Mannich, *Chem.-Ztg.*, 1909, **33**, 1167.

[42] Clark, U.S.P. 1,293,039, 1919.

[43] E. Grünsteidl, *Mikrochemie*, 1933, **13**, 183.
[44] N. R. Dhar, "The Chemical Action of Light" (London: Blackie & Sons, Ltd., 1931).
[45] W. V. Bhagwat, *Jour. Ind. Chem. Soc.*, 1934, **11**, 5.
[46] H. L. J. Bäckström, *Naturwiss.*, 1933, **21**, 251.
[47] R. Lassé, *Helv. phys. Acta*, 1932, **5**, 92.
[48] R. W. Wood, "Physical Optics," 1914, p. 15.
[49] Gaumont and Soc. des Établissements, E.P. 351,029, 1929.
[50] R. B. Withrow and Gen. Development Labs. Inc., U.S.P. 1,938,734, 12/12/33.
[51] W. Holthoff, *Zeit. anal. Chem.*, 1933, **91**, 263.
[52] A. H. Taylor, *Jour. Opt. Soc. Amer.*, 1934, **24**, 192; B. K. Johnson, *Nature*, 1934, **134**, 216.
[53] P. W. Danckwortt and E. Jürgens, *Arch. Pharm.*, 1934, **272**, 713.
[54] P. Carnot and R. Coquoin, *Compt. rend. Soc. Biol.*, 1926, **94**, 850.
[55] L. Szilard and T. Chalmers, *Nature*, 1934, **134**, 494.
[56] Tokyo Denki K. K., Jap. Pat. 101,817, 1/7/33.
[57] C. F. Goodeve, *Nature*, 1934, **134**, 416.
[58] C. Fabry, *ibid.*, 1934, **134**, 736.
[59] J. Saidman and Dufestel, *Compt. rend.*, 1926, **182**, 1173; 1933, **196**, 1537.
[60] A. Schober, *Nature*, 1934, **134**, 898.
[61] H. J. Taylor, *ibid.*, 1935, **135**, 35.
[62] W. de Groot, *ibid.*, 1935, **135**, 68.
[63] E. J. Bowen, *Jour. Chem. Soc.*, 1935, 76.
[64] W. W. Coblentz and J. R. Stair, *Jour. Res., Bureau of Standards*, Dec., 1934.
[65] B. K. Vaidya, *Jour. Univ. Bombay*, 1934, **3**, 141.
[66] C. A. Becker, *Sprechsaal Keram.*, 1934, **67**, 137.
[67] J. Doetsch, *Anal. Fis. Quim.*, 1934, **32**, 65.
[68] F. Schlemmer and F. Maier, *Deut. Molkerei-Ztg.*, 1934, **55**, 1477.
[69] C. Joseph, U.S.P. 2,038,114, 21/4/36: E.P. 410,170, 1934.
[70] DuPont and Nemours, U.S.P. 2,062,179, 24/11/36.
[71] I.G. Farbenind., Anm. I., 52,047; E.P. 457,708, 1936; F.P. 804,582, 1936.
[72] O. Gerngross, E.P. 459,335, 1936.
[73] O. Behaghel, Anm. B., 166, 30/30 i.
[74] I.G. Farbenind., Can. Pat., 346,197; E.P., 436,891, 1935; *Chem. Zentr.*, 1935, **106**, ii, 1807.
[75] F. E. Stockelbach, U.S.P. 2,128,334.
[76] I.G. Farbenind., E.P. 490,516, 1938.
[77] I.G. Farbenind., Swiss P. 198,429.
[78] W. V. Bhagwat, *Bull. Acad. Sci.*, U.P. 1932, **2**, 67.
[79] J. A. Benkiser and F. Draisbach, E.P. 436,426, 21/2/35.
[80] K. Fuwa, U.S.P. 1,971,309, 1934; U.S.P. 1,983,359, 1934.
[81] C. Dreyfus, U.S.P. 2,002,082, 21/5/35.
[82] E. Gilles, *Compt. rend.*, 1936, **202**, 968.
[83] G. Heyne and M. Schön, *Angew. Chem.*, 1936, **49**, 784.
[84] D. W. Dana, *Rev. Sci. Instr.*, 1935, **6**, (ii), 417.

ADDITIONAL REFERENCES.

Anon, *Nature*, 1935, **136**, 60 (Glass filter transmitting at 3650 Å. and no visible light).

H. P. Hood, U.S.P. 1,964,321, 26/6/34 (Glasses containing silica, boric acid, and sodium, for metal vapour lamps).

CHAPTER IV.

THE MEASUREMENT OF THE INTENSITY OF SOURCES OF ULTRA-VIOLET LIGHT.

THE method selected for the measurement of the intensity of the ultra-violet light from any particular source depends on the purpose for which the light is to be used ; therapeutic work and fluorescence analysis, for example, are concerned with different spectral regions. Sources of ultra-violet light vary in spectral characteristics and peculiarities ; quartz mercury-vapour lamps, for instance, deteriorate with use, and the decrease in energy emission is not uniform throughout the spectral range.

Any effect produced by ultra-violet light may be used to measure it, and such effects may be photochemical, photoelectrical, photogenic, germicidal or physiological. These divisions are not strictly independent of one another, but it is not the intention to discuss the various methods of measurement in great detail, except where it appears to be desirable to do so, because a few particular methods have become to some extent standardised by virtue of fairly wide use by different workers.

Chemical Methods.—Chemical methods depend on the measurement of the extent of a chemical reaction which occurs under the influence of ultra-violet light. The reaction chosen should be reproducible and easy to carry out, it should respond readily to analytical tests, and it should be capable of measuring accurately the relative strengths of various lamps or the change in energy-emission of any one lamp at various periods ; the reaction should involve only one phase, and the photochemical efficiency should be known for the wave-lengths concerned (*cf.* Forbes [41]). If the lamp is to be used for therapeutic practice, the method should be capable of giving an accurate comparison between the relative intensities of waves of short wave-length emitted by various lamps, and of solar radiation under varying

meteorological conditions, and the results should be easily interpretable in terms of physiological effect. Unfortunately, no one method has proved completely satisfactory, as each chemical reaction is influenced by radiations only over a relatively restricted spectral range. There are, however, a number of methods which satisfy most requirements if the technique is standardised and followed carefully.

The Bordier actinometer consists of a piece of filter-paper moistened with a 20 per cent. solution of potassium ferrocyanide. On exposure to ultra-violet light, this paper becomes discoloured owing to the formation of iron, and the paper is then compared with a set of standard colours. A paper to be used in a similar way, and impregnated with *o*-nitrobenzaldehyde and litmus, is produced by the Eastman Kodak Co.[38]

According to Schall,[1] paper prepared with 0·66 *N p-phenylene-diamine nitrate* is sensitive to radiation of wave-length shorter than 3130 Å., and such paper was used by Mott and Bedford to compare various arc sources. A solution is made containing 1 grm. of *p*-phenylenediamine, 2 c.c. of nitric acid (sp. gr. 1·21), and 3 c.c. of water; blotting paper is impregnated with this liquid and is then dried fairly quickly in the oven. If the paper is allowed to dry under ordinary conditions it blackens and becomes useless. On exposure to ultra-violet light the paper becomes green and finally turns to a metallic-brown colour.

Phototropic Dyes.—A. Webster, L. Hill and Eidinow [2] introduced a method in which the intensity of ultra-violet light is measured in terms of the fading of a solution of *methylene blue* in acetone, the colour being then compared with a standard set of solutions of methylene blue of various concentrations. The dye, which is decolorised by reduction, acts as a hydrogen acceptor in the complex processes (formation of acetic and formic acids, etc.) which occur on irradiation.

For the operation 5·8 c.c. of a 0·1 per cent. solution of the dye are added to 30 c.c. of acetone, and the solution is then diluted with water to 100 c.c. Before use 2 parts of this solution are mixed with 1 part of acetone, and are placed in a quartz tube which is then exposed under the lamp at a fixed and measured distance from it. After a predetermined time the colour of the solution is compared with the colours of a set of standard solutions, and the results are expressed in empirical units of bleaching. The

action of the light is not uniform, the portion of the solution near the lamp being bleached more rapidly than the rest, which is protected by the absorbing power of the intermediate layer of liquid. The test is rather slow in sunlight, but is fairly rapid with the mercury or carbon arcs. Methods involving the comparison of fading effects are not very accurate, but this method has the advantage that no complicated apparatus and few chemicals are required. A. E. Gillam and R. A. Morton [3] have shown that the rays of 2000 to 2800 Å. account for 80 per cent. of the change, rays of 2800 to 3250 Å. for 6 per cent., and rays of 3250 Å. and longer wave-lengths for the remainder. Air must be excluded, as it also causes fading,[26] and allowance should be made for the effects of rays of longer wave-lengths by a blank exposure in a glass tube.[48] The temperature-coefficient is 1·5 for every 10° C. The methylene blue method may also be used in conjunction with photometric measurement (see Nurnburger and Arnow,[31] p. 45).

H. S. Mayerson [49] uses the number of units of standard acetone-methylene blue solution, bleached in one hour by a medical lamp, multiplied by 700 ergs per sq. cm. per sec., to obtain the amount of energy available for antirachitic purposes. It should also be noted that the fading shown by the methylene blue is reversible in the dark [50] (see p. 337), and therefore, readings should be made as soon as possible after irradiation. The acetone-methylene blue method has also been examined by H. Schreiber [51] and Friedrick and Bender.[52]

Another method involving the use of dyes has been developed by the I.G. Farbenind., A.-G.[4] Solutions which contain phototropic compounds, such as the leucocyanines, carbinols or the sulphurous acid compounds of crystal violet, malachite green, fuchsin, etc., and which also contain small quantities of substances tending to counteract photochemical change, are exposed under the lamp. After a definite time these irradiated liquids are compared with standard solutions of the dyes themselves, and the results are thus given in empirical terms of colour intensity. The substances added to retard the photochemical effect are potassium cyanide or hydroxide, and sulphurous acid. This method is claimed to give good results in the region of the spectrum between 2800 and 2500 Å., and is therefore of particular use with the tungsten arc and, according to E. Weyde and W. Frankenburger,[29] for lamps required for therapeutic purposes.

T. Hanzawa [5] and D. Sachs [27] have recently suggested the use of a dilute solution of *ammonium molybdate* in dilute hydrochloric acid containing a small quantity of ethyl alcohol or formic acid, as a suitable liquid for use in actinometers. On exposure to sunlight or ultra-violet light this solution develops a blue colour, which may be compared with standard colours in the usual way.

Another method due to Mackenzie and A. King [6] (see also C. H. Harvey [28]) is based on the *decomposition of carbon tetrachloride* into chlorine and hexa-chloroethane when irradiated by ultra-violet light, a drop of a 10 per cent. solution of potassium iodide being added to the irradiated liquid. The iodine liberated gives a solution, the violet colour of which is compared with a standard set of solutions made by dissolving known amounts of iodine in carbon tetrachloride. Massol and Faucon have shown that it is difficult to obtain spectroscopically-pure carbon tetrachloride, as the carbon disulphide present in the commercial tetrachloride is hard to remove, and Mackenzie and King [6] emphasise the necessity of using medicinally-pure carbon tetrachloride which is more active towards light than the commercial product. This reaction is very sensitive to a decrease in output of the quartz mercury-vapour lamp, but little or no decomposition is obtained with alpine sunlight. As the photochemical rays valuable in actinotherapy are between 2650 and 3050 Å., the method is of little value for the comparison of light sources for this purpose.

Uranium Salts-Oxalic Acid.—In 1907 Bacon [7, 8] showed that many aliphatic acids are photochemically decomposed in the presence of uranium salts, and that alkaloids have a retarding influence on this reaction. With oxalic acid he found that the rate of decomposition increases with increase in the concentration of the uranium salt, the decomposition corresponding with 92 per cent. of the oxalic acid. Thus 0·1 grm. of uranium acetate can catalyse the decomposition of up to 1·5 grm. of oxalic acid. Bacon [8] further showed that the nitrate has the same effect as the acetate, that the speed of the reaction is practically the same at 100° as at 30° C., and that there is no reaction if the solution is alkaline. J. H. Matthews and L. H. Dewey [9] exposed a 0·1 N solution of oxalic acid, at 25° C., 10 cm. from a lamp, and noted that uranium acetate, nitrate and sulphate all accelerate the reaction in proportion to the amount of uranium present. In

1924 W. C. Holmes [10] suggested the reaction as a means of measurement of the light used for fading tests.

Finally the reaction has been carefully examined by Moss and Knapp,[11] who maintain that a mixture of oxalic acid and uranium acetate solutions gives more accurate results than the acetone-methylene blue method. The mixture is placed in a flat-bottomed dish with parallel upright sides, 8 cm. deep, which is coated on the outside with a layer of red paint, and then with three coats of white paint, so as to eliminate light or heat from outside sources (the " Uroxameter "). The dish is then covered with a silica plate 2·5 mm. thick, and placed on a table which can be rotated slowly. Moss and Knapp recommend a standard distance of 6·5 ins. from the lamp in order to reduce heating effects, but as they themselves have shown, this has but little influence on the reaction. After an exposure to the rays from the lamp for a period of 30 minutes, the oxalic acid is titrated with 0·1 *N* potassium permanganate solution, the same amount of the unexposed solution being also titrated so as to serve as a control; the amount of oxalic acid decomposed is then expressed as a percentage (the " *Uroxameter Value* " of the lamp). The test is independent of the amount of water present in the oxalic acid solution, of the temperature, or of the speed of rotation of the turn-table. However, the strong " twenty strength " solution used for carbon arcs deposits a precipitate which may affect the results. The absorption spectrum of the solution alters with the concentration of the uranium salt and therefore, as may be expected, the speed of the reaction varies correspondingly with a change in concentration of the uranium salt.

A somewhat similar method is that of W. T. Anderson and F. W. Robinson.[12] They dissolve 6·3 grms. of crystalline oxalic acid and 4·2 grms. of crystalline uranium sulphate in water and dilute the solutions to 1 litre, 25 c.c. being irradiated for a predetermined time at a definite distance from the light source, and then titrated with 0·1 *N* potassium permanganate solution. The results are expressed as mgrms. of oxalic acid decomposed, and this is taken as representing the photochemical effect of the lamp. It has been found that 25 to 30 per cent. of the decomposition is due to the rays of wave-lengths of 3250 Å. or over, and 40 per cent. to waves of 2800 Å. or over, while rays between 2700 and 3250 Å. account for about 20 per cent. of the change.

W. G. Leighton and G. S. Forbes,[13] and Freer and Gibbs,[14] have thoroughly tested the uranyl-oxalate method, and have determined its temperature-coefficient between 15° and 30° C. for 9 monochromatic wave-lengths between 3660 and 3130 Å., and Forbes and Heidt [46] have investigated the effects of variations in concentration of the reagent. Atkins and Poole [40] have applied it successfully to the measurement of daylight. According to N. E. Gordon and D. A. Wilson [53] the complex which undergoes unimolecular decomposition is $UO_2(C_2O_4)_2''$, and the source of quadrivalent uranium is $UO_2C_2O_4$. H. S. Mayerson [49] suggests that the intensity of irradiation by rays of wave-length between 2900 and 3900 Å. may be expressed as ergs per sq. cm. per sec. by multiplying the number of mgrms. of oxalic acid decomposed per sq. cm. of the surface of liquid in the Uroxameter in 30 minutes by 18,300.

The photochemical decomposition of some organic acids has been studied by W. C. Pierce and Glen Morey,[15] who note that these photochemical decarboxylations are characterised by low temperature-coefficients and low light absorption, while the quantum efficiency is very near unity. If, therefore, there are no other absorbing groups present to interfere with the reaction, this method should also be suitable for the study of the intensity of a source of ultra-violet light. B. J. Dain and E. S. Pusenkin [54] used hydrogen peroxide and trichloroacetic acid in an actinometer.

The decomposition of potassium nitrate into nitrite under the influence of ultra-violet light has been investigated fully by A. E. Gillam and R. A. Morton,[16] although the reaction itself has been known for many years.[39] It was shown by Browning and Russ [17] that the bactericidal rays fall between 2000 and 2970 Å., with a maximum, according to F. L. Gates,[18] between 2600 and 2700 Å. The rays between 2000 and 2800 Å. account for more of the decomposition of potassium nitrate than those falling in the region of selective absorption, *i.e.* between 2800 and 3300 Å., so that it is apparent that the photolysis of potassium nitrate is very similar, so far as concerns spectral distribution, to bactericidal effects. Warburg [30] has observed that the reaction proceeds most rapidly in alkaline solution, and Villars,[19] who followed the decomposition by means of a modification of Warburg's iodimetric method, fixed the optimum *p*H value at 9·0. To the irradiated solution of potassium nitrate is added some potas-

sium iodide solution, and the iodine liberated is titrated with sodium thiosulphate solution. Unduly long exposures are, however, required in order to give reasonably large burette-readings, and Gillam and Morton therefore used the delicate Griess-Ilosvay reaction to measure the amount of nitrite formed on irradiation.

A buffered solution is made up as follows: 10·1 grms. of potassium nitrate are dissolved in 250 c.c. of water to which potassium chloride and boric acid have been added so that the solution is 0·2 *M* with respect to both. To this solution is added 160 c.c. of a 0·2 *N* solution of sodium hydroxide, free from carbonate, and the whole is then diluted to 1 litre with carbon dioxide-free water, and stored in a brown glass bottle. The nitrite reagent is made by mixing a solution of 0·1 grm. of α-naphthylamine in a little hot water with a solution of 0·5 grm. of sulphanilic acid dissolved in 150 c.c. of dilute (1 : 4) acetic acid, the mixture being then diluted to 300 c.c. with dilute acetic acid. This reagent, also, should be kept in dark glass bottles out of contact with the air, and should be discarded if a pink colour develops. A quartz tube (6 × 0·75 ins.) containing the buffered nitrate solution is then placed 12 ins. from, and at right angles to, the axis of the lamp.

After a predetermined time, 10 c.c. of the solution are pipetted into a Nessler cylinder, 2 c.c. of glacial acetic acid and the nitrite reagent are then added, the solution then being diluted to 40 c.c. with water. A standard is made up in a similar tube from distilled water, glacial acetic acid, nitrite reagent and 1 or 2 c.c. of a standard solution of sodium nitrite. The two solutions are then set aside for half an hour to allow the colour to develop, and are then compared. It is best to avoid too long an exposure, as this gives a concentration of the dye which is too high for accurate comparison. The standard nitrite solution is made by adding a slight excess of sodium chloride to a solution of 1·539 grms. of silver nitrite in water, the precipitated silver chloride being removed by filtration, and the combined filtrate and washings diluted to 1 litre with distilled water. This solution is again diluted to 1 : 50 before use, so that the final liquid is 0·0002 *M* with respect to sodium nitrite.

O. Baudisch and F. Benford [64] suggest the use of potassium nitrate, together with an ice-cold solution of 2·7-diamino fluorene

hydrochloride, to measure the intensity of ultra-violet light between 2650 and 3341 Å., the solution being irradiated in a sealed tube. With the photochemical decomposition of the potassium nitrate the 2.7-diamino fluorene hydrochloride is oxidised to a blue compound.

Comparison of Chemical Methods.—In an important paper Hymas [24] has compared a number of the above methods, and he demonstrates that each reaction has a maximum sensitiveness for one particular region of wave-lengths. The methods used were substantially the same as those already described, except that in the case of the potassium iodide method 0·1 *N* potassium iodide containing 0·1 per cent. of starch was exposed, and the blue colour was matched against the glasses of the Lovibond tintometer. It should be noted, incidentally, that even potassium iodide solution exposed to daylight on the laboratory shelf decomposes to some extent with the formation of free iodine. Hymas shows his results most strikingly in the form of curves relating wave-length and photochemical activity, and they are also summarised in Table 3. It has been found [55-58] that similar curves (for solar radiation) are obtained by the potassium nitrate and potassium iodide methods, and that consistent results over a period of 10 years are obtainable by the potassium iodide method despite its several disadvantages.

TABLE 3.

Reagent.	Wave-length Range. ($m\mu$.)	Optimum Wave-length. ($m\mu$.)
Potassium iodide	220-320	255
Potassium nitrate—nitrite	220-400	255
Acetone-methylene blue	250-500	275
Oxalic acid—uranyl sulphate	220-500	260 and 325 (min. at 280)

Physical Methods.—Chemical methods, though convenient, are necessarily founded on an empirical basis, and if absolute results are required physical methods must be used. Fortunately, this is not frequently the case in connection with analytical work, and the expensive apparatus involved is therefore seldom required.

Spectrophotometric Methods are usually based on the principle described by Judd Lewis (see his work on paper, p. 341); it is

not necessary, however, to use photographical methods unless a permanent record is required, and the camera may be replaced by any form of photometer, the Judd Lewis sector type having much to commend it. It is outside our scope to deal in detail with photometers, but reference should be made to the procedure of C. E. Nurnburger and L. E. Arnow [31] since this is combined with the methylene blue reaction (p. 38). The Judd Lewis sector photometer spectrograph is used in this case to measure the ultra-violet and visible absorption spectra of an aqueous solution of methylene blue before and after irradiation with the source of light. The changes are greatest at high *p*H values and for wave-lengths below 2700 Å., so that the method is unsuitable for physiologically-active radiations. A simple fluorophotometer is also described by B. T. Squires and J. H. Jeffree.[25]

The *Meethem* ultra-violet daylight photometer is a combination of integrating spheres and a photographic method of measurement, using a silvered quartz ultra-violet filter and a gelatine wedge. It has been used successfully for the measurement of ultra-violet light in solar radiation,[59] and it is simple to work, low in cost, and the solar radiation from the whole hemisphere of the sky is integrated, the ultra-violet radiation being measured over a narrow band of wave-lengths.

In the Ashworth ultra-violet ray meter [60] a paper wedge overlapping 10 strips of paper is used to obtain graduations in the recordings on photographic paper, and this has been replaced by one worker by varying thicknesses of wire gauze.[55] This prevents alteration in the transmission factor of the wedge, which is liable to take place with the paper wedge owing to the effect of humidity or of discoloration by sunlight.

The Photo-Electric Cell is now regarded as a useful adjunct for photometric work, since the results obtained are independent of the personal factor.[48] As is well known its use depends on the phenomenon first studied by Hallwachs and by Hertz, namely, that a charged plate loses its potential when irradiated, and the resulting current is a measure of the intensity of the radiation responsible. In the modern cell the material of the plate may be so selected as to ensure sensitiveness to certain ranges of wave-lengths.

H. D. Griffiths and J. S. Taylor [20] have described such an apparatus. A metal plate irradiated by rays of wave-length less

than a certain critical value emits electrons, so that if the plate is first given a negative charge, this charge gradually leaks away during irradiation. The loss of the charge is conveniently followed by means of a gold-leaf electroscope connected with the metal plate, since the rate of fall of the gold leaf in the electroscope depends on the rate of leakage of the charge from the plate, which in turn depends on the intensity of the radiation falling on it. Griffiths and Taylor used a cadmium photo-electric cell which is sensitive to radiations of wave-lengths between 3500 and 2000 Å. The apparatus gives good results, with an error of less than 5 per cent., and is rapid in use.

For a summary of the characteristics of photo-electric cells and methods of use, the papers of Coblentz and his co-workers [21-23, 32] are to be recommended; these also contain a valuable bibliography of the subject.

The method used by them [32] involves a balanced amplifier, a micro-ammeter and a photo-electric cell responding to a selective wave-length and calibrated by means of a thermopile, and it gives a linear response after the elapse of preliminary period of about 10 minutes to ensure a steady zero; it is, however, applicable chiefly to therapeutic work. W. T. Anderson [33] has used the Weston "Photronic" cell, and A. Chevallier and P. Chabre [34] have combined the principles of spectrophotometry and photo-electricity for a similar purpose; F. H. Cohen [47] prefers a selenium cell. A cadmium photo-electric cell is suggested by M. Bender [65] for the measurement of ultra-violet light in sunlight, and an integrating solarimeter consisting of a Moll pattern thermopile coupled with a sensitive milliampere-hour indicator, has also been used to record the total solar radiation. For the calibration of photo-electric intensity meters W. W. Coblentz and R. Stair [61] recommend the quartz mercury arc lamp as the source of light.

Photographic Methods may well be regarded as another, if less important, category of physical methods, and in this may also be grouped methods in which the nature and intensity of the fluorescence produced by the source of ultra-violet light are used to measure the intensity of the exciting radiation. An example of a combination of both principles is the process suggested by W. D. Fleming,[35] who coated a photographic emulsion with mineral oil, the fluorescence produced by the latter in ultra-violet light being recorded as an image on the final plate. P. W. Danckwortt and E. Jürgens [36] used fluorescent screens to measure the intensity

of ultra-violet light in connection with work on filters, and S. E. Sheppard and L. W. Eberlin [37] have patented a film in which is incorporated compounds of molybdenum, tungsten or thallium, an oxidisable substance (*e.g.*, glucose), and a slight excess of citric or hydrochloric acid ; radiation of wave-length, 4000 Å. or less, turns it from blue to white. Numerous other devices, including a portable meter, are given in the references.[42-44]

The zinc sulphide method described by Clark [62] is not strictly a photographic method, but depends on the decomposition and consequent darkening of zinc sulphide when exposed to ultra-violet light. Mayerson [49] considers it to be simple, rapid and efficient, but that a " reflection factor " should be used to measure the degree of darkening, as colour charts are unsatisfactory. The change is irreversible and is sensitive to radiation of wave-lengths 2900 to 3500 Å. (the antirachitic range), and under the specified conditions the darkening in colour is proportional to the intensity of the light. A change of one unit in this method is stated by Mayerson to be equivalent to the emission by the lamp of 735,000 ergs per sq. cm. by rays having wave-lengths between 2900 and 3500 Å.

J. S. Owens [63] points out that the optical properties of wedges are not often permanent, reproducible or constant, and to overcome these objections he uses a series of blackened tubes having plates pierced by orifices at each end. In the bottom end the diameter of the orifice is 3/16 in. in every case, but those in the top plates vary from 0·7 to 10 mm. in diameter. A No. 14 Chance filter is placed over the top, so that only radiations of wave-length 3000 to 4000 Å. are transmitted, and the bottom ends of the tubes rest on a piece of Kodatone paper. On exposure to light a number of spots are produced, the density of which is proportional to the area of the top hole. The scale is so adjusted that a whole day's exposure on the brightest and longest day just produces a spot under the tube having the smallest orifice, whereas the dullest and shortest day results in a similar effect under the tube with the largest aperture.

The Filter Method, for the measurement of the intensity of ultra-violet light, does not appear to have attracted quite the attention that it deserves, although P. Dubouloz [45] applied it successfully to the measurement of rays of short wave-length. A layer of sodium salicylate 0·05 mm. thick completely absorbs light below 3450 Å., and the intensity of the fluorescence is therefore

a measure of the intensity of the original radiation, the relation being almost linear between 3450 and 2600 Å. A similar principle was used by A. Chevallier and P. Chabre,[34] who allowed the light to fall on a fluorescent substance and measured the intensity of the resulting glow with a photo-electric cell.

REFERENCES.

[1] Schall, *Chem. Ztg.*, 1910, 34, 267.
[2] A. Webster, L. Hill and Eidinow, *Brit. Jour. Radiology*, 1924, **22**, 335.
[3] A. E. Gillam and R. A. Morton, *J. Soc. Chem. Ind.*, 1927, **46**, 417.
[4] I.G. Farbenind., A.-G., B.P. 296,198.
[5] T. Hanzawa, *J. Agric. Chem. Soc. Japan*, 1930, **6**, 1093.
[6] Mackenzie and A. A. King, "Practical Ultra-Violet Light Therapy" (Benn), 1926.
[7] R. F. Bacon, *Philippine Jour. Sci.*, 1907, **2**, 129.
[8] R. F. Bacon, *ibid.*, 1910, 5, 281.
[9] J. H. Matthews and L. H. Dewey, Eighth Intl. Congress of Applied Chem., N.Y., 1912, **20**, 247 ; *J. Phys. Chem.*, 1913, **17**, 216.
[10] W. C. Holmes, *American Dyestuff Rep.*, 1924, **13**, 188, 197.
[11] Moss and Knapp, *Jour. Soc. Chem. Ind.*, 1925, 44, 453 ; *Brit. Jour. Actin.*, 1927, Jan.
[12] W. T. Anderson and F. W. Robinson, *Jour. Amer. Chem. Soc.*, 1925, **47**, 718.
[13] W. G. Leighton and G. S. Forbes, *ibid.*, 1930, **52**, 3139.
[14] Freer and Gibbs, *Jour. Phys. Chem.*, 1912, **16**, 709.
[15] W. C. Pierce and Glen Morey, *Jour. Amer. Chem. Soc.*, 1932, **54**, 467.
[16] A. E. Gillam and R. A. Morton, *Jour. Soc. Chem. Ind.*, 1927, **46**, 415.
[17] Browning and Russ, *Proc. Roy. Soc., London*, 1917, **90**, 33.
[18] F. L. Gates, *Jour. Gen. Physiol.*, 1930, 14, 31.
[19] Villars, *Jour. Amer. Chem. Soc.*, 1927, **49**, 326.
[20] H. D. Griffiths and J. S. Taylor, *Lancet*, 1925, 1205.
[21] W. W. Coblentz, *Bur. Stand. Sci. Paper*, No. 349.
[22] W. W. Coblentz, *Bull. Bur. Stand.*, 1908, 4, 404.
[23] W. W. Coblentz, *ibid.*, 1912, 9, 7.
[24] C. F. Hymas, *Quart. Jour. Pharm. and Pharmacol.*, 1929, **2**, 281.
[25] B. T. Squires and J. H. Jeffree, *Jour. Sci. Inst.*, 1928, **5**, No. 9.
[26] M. Qureshi and N. A. Tahir, *Jour. Phys. Chem.*, 1932, **36**, 2670.
[27] D. Sachs, *J. Chim. Phys.*, 1932, **29**, 474.
[28] E. H. Harvey, *Amer. Jour. Pharm.*, 1933, **105**, 36.
[29] E. Weyde and W. Frankenburger, *Trans. Faraday Soc.*, 1931, **27**, 561.
[30] Warburg, *Sitzb. Preuss. Akad. Wiss.*, 1918, 1228.
[31] C. E. Nurnburger and L. E. Arnow, *Jour. Phys. Chem.*, 1934, **38**, 71.
[32] W. W. Coblentz, R. Stair and J. M. Hogue, *Bur. Stand. Jour. Res.*, 1931, **8**, 759 ; 1934, **12**, 231 ; *Bur. Stand. Res. Paper*, No. 450, 1932.
[33] W. T. Anderson, *Rev. Sci. Inst.*, 1934, **5**, 127.
[34] A. Chevallier and P. Chabre, *Biochem. Jour.*, 1933, **27**, 298.
[35] W. D. Fleming, *Philippine Jour. Sci.*, 1933, **50**, 185.
[36] P. W. Danckwortt and E. Jürgens, *Arch. Pharm.*, 1934, **272**, 713.
[37] S. E. Sheppard, L. W. Eberlin and Eastman Kodak Co., U.S.P. 1,934,451, 7/11/33.
[38] Eastman Kodak Co., U.S.P. 1,878,684, 20/9/32.

[39] Thillard, *Ber.*, 1907, 40, 4914; M. Lombard, *Compt. rend.*, 1910, **150**, 227; Berthelot and Gaudechon, *ibid.*, 1911, **152**, 522; Baudisch, *Ber.*, 1911, 44, 1009; Dhar and Sanzal, *Jour. Phys. Chem.*, 1925, **29**, 926.
[40] W. R. G. Atkins and Poole, *Sci. Proc. Roy. Dublin Soc.*, 1929, **19**, 321.
[41] G. S. Forbes, *Jour. Phys. Chem.*, 1928, **32**, 482.
[42] G. S. Forbes, L. J. Heidt and L. W. Spooner, *Rev. Sci. Inst.*, 1934, **5**, 253 (Comparison of optical methods).
[43] D. H. Follett, *Proc. Phys. Soc.*, 1934, **46**, 499 (Photo-electric method, with rotating sector).
[44] A. H. Taylor, *Jour. Opt. Soc. Amer.*, 1933, **23**, 60; 1934, **24**, 133 (Portable apparatus).
[45] P. Dubouloz, *Comp rend.*, 1933, **196**, 1221.
[46] G. S. Forbes and L. J. Heidt, *Jour. Amer. Chem. Soc.*, 1934, **56**, 2363.
[47] F. H. Cohen, *Rec. Trav. Chim.*, 1935, **54**, 133.
[48] Department of Scientific and Industrial Research. "Investigation of Atmospheric Pollution. Report for year ending 31st March, 1934." (London, April, 1935.)
[49] H. S. Mayerson, *Amer. J. Hygiene*, 1935, **22**, 106.
[50] Anon, *21st Report Dept. Sci. Ind. Res.*, 1936.
[51] H. Schreiber, *Meteorol. Z.*, 1933, 399.
[52] Friedrich and M. Bender, *ibid.*, 1930, 285.
[53] N. E. Gordon and D. A. Wilson, *J. Amer. Chem. Soc.*, 1936, **58**, 67.
[54] B. J. Dain and E. S. Pusenkin, *Ber. Ukrain. Fortschr. physik. Chem.*, 1935, **4**, 75.
[55] Anon, *Report of City Analyst, Salford, for* 1936.
[56] Anon, *Analyst*, 1927, **52**, 641.
[57] J. E. Moss and A. W. Knapp, *ibid.*, 1929, **54**, 334.
[58] Anon, *ibid.*, 1931, **56**, 314.
[59] Anon, *Annual Report Dept. Sci. Ind. Res.*, 1938.
[60] Anon, *Analyst*, 1933, **58**, 690.
[61] W. W. Coblentz and R. Stair, *J. Res. Nat. Bur. Stds.*, 1936, **16**, 83.
[62] J. H. Clark, *Analyst*, 1929, **54**, 493.
[63] J. S. Owens, *Lancet*, 1935, **229**, 589.
[64] O. Baudisch and F. Benford, *Naturwiss.*, 1936, **24**, 361.
[65] M. Bender, *Physik. Zeit.*, 1936, **37**, 107.

ADDITIONAL REFERENCES.

E. V. Schpolski and N. I. Kolesnikova, *J. Phys. Chem. U.S.S.R.*, 1934, **5**, 1199; *ibid.*, 1935, **6**, 133 (The course of the Eder reaction is dependent on intensity of radiation).

Committee on Nomenclature and Standards, *Trans. Illum. Eng. Soc. Amer.*, 1935, No. 7, 30.

W. T. Anderson, *Quartz Lamp*, 1935, 46 (Photo-electric cell and rheostat used to obtain constant output).

R. H. Müller and G. E. Shriver, *Rev. Sci. Inst.*, 1935, Jan. (Photo-electric cell charging a condenser which operates an automatic counter).

H. H. Bagnall, *Analyst*, 1929, **54**, 101 (Strength of sunlight).

J. H. Clark, *Amer. J. Hygiene*, 1929, **9**, 646 (Zinc sulphide method for sunlight).

Anon, *Analyst*, 1933, **58**, 691 (Ashworth method which uses tissue paper wedge described).

Anon, *Trans. Illum. Eng. Soc.* (*Amer.*), 1935, no. 7, 30 (Evaluation of ultra-violet radiation).

CHAPTER V.

METHODS AND TECHNIQUE OF FLUORESCENCE ANALYSIS.

IT seems desirable to devote some space to a discussion of the methods employed by different workers for the examination of fluorescent substances, as the varying results sometimes obtained by different workers for the same substance are often due to lack of precision in defining the technique. Certain of the more specialised methods are described more fully in the appropriate chapters, as it is not intended here to describe in great detail all the types of apparatus employed, but merely to indicate the general methods of examination of a fluorescent substance and some of the pitfalls encountered in such work. It should be mentioned at this point that some of the pioneer work on ultra-violet light as an aid to analysis is due to R. W. Wood,[52] who so long ago as 1911 indicated some likely applications and developed apparatus and technique for carrying them out; "Wood's glass" filters are now of course well known and indispensable to every worker (see also J. Grant [53]). The fluorescence of the eye has been suggested as a possible source of observation of fictitious colour values by Grünsteidl (p. 31), but there appears to be no experimental evidence that it can cause appreciable errors.

The choice of the lamp rests with the investigator, but to-day most workers prefer the quartz mercury-vapour lamp, with screens of "Wood's glass" or filters allowing rays of wave-length 3650 Å. to pass. Other workers have used arc lamps with iron, tungsten or carbon electrodes. The various lamps are compared and described in Chapter II., and filters are described in Chapter III. Whichever lamp is used, however, the spectrogram of the filter should first be obtained if the results are to be comparable with those of other workers.

Containers.—For many industrial purposes the substance to be examined may be placed in a non-fluorescent glass or porcelain dish, and a direct observation made under the lamp. Containers

made from some heavy dark papers, such as black filter-papers, are very effective for solids which give a white fluorescence, but it is preferable to use quartz where possible. Petri dishes may be covered with cellophane in order to keep out contamination, and yet allow the passage of ultra-violet light. In some cases variations in the colour of the fluorescence are obtained as the distance from the sample to the lamp is altered.

Labels on containers or samples may be written with the fluorescent crayons recently introduced,[105] which contain anthracene or uranium salts with chalk and plaster of paris ; and J. A. Radley has used an ink made by dissolving quinine sulphate in a 0·25 per cent. solution of Ink Blue AS or, preferably, an aqueous solution of Eosine Y125, both of which can be read in the dark room under the lamp, and in daylight.

State of the Sample.—The substance itself may be in the massive, or powdered state, or in solution, and show differences in fluorescence colour accordingly. Thus, some substances do not fluoresce when solid but do so when in solution, and *vice versa*. *The fineness of division* of a powder may also influence the intensity or colour in certain cases ; in general the fluorescence appears much brighter if the substance is in the powdered state, although in cases of extreme subdivision it may fade again ; furthermore, it often deepens or changes in colour when the substance is in the form of a voluminous precipitate.

If a substance is examined in *the vapour state* the intensity of the fluorescence may be influenced by the vapour-pressure, or by the vapour-density. An example of this is given by Niewodniczański [1, 2] in a short paper on the results of his work with mercury vapour. The vapour at a temperature of 240° C. was illuminated by ultra-violet light, and it was found that when the density was altered there was a corresponding change in the intensity of the fluorescence. The vapour state is, however, rarely used for ordinary analytical examinations.

Solvents.—These may be of great importance, as it is a matter of some difficulty to obtain them free from fluorescence ; the colours usually observed will be found on page 303. Wawilow and Tummermann [64] found that when a blue fluorescence is obtained, it usually has a maximum at 4000 to 4200 Å., and this is ascribed to gases taken up from the air. The fluorescence of many solvents is certainly due to the presence of impurities (see p. 54), and

a stringent purification process will therefore eliminate it. Thus, glycerol used as a mounting medium in fluorescence microscopy must be treated with animal charcoal, since ordinary distillation has been found to be without effect.[60] K. Weber and M. L. Savić [65] have also shown that the nature of the solvent may influence the effect of foreign substances on the extinction of fluorescence (see below and p. 290). These considerations must be taken into account when the fluorescence is being used as a measure of the concentration of certain solids in suspension (*e.g.*, pharmaceutical zinc oxide, see J. Eisenbrand and G. Siewert [63]). In the case of some dyes (see p. 381) the intensity of the fluorescence is a function of the viscosity of the solvent, except at infinite dilution,[95, 140] but in other cases (*e.g.*, quinine sulphate, aesculin and rhodamine-B in water, ethyl alcohol or glycerol) no such relationship has been established.[147]

Dilution.—The concentration of the solution is another important factor, although, naturally enough, this affects the intensity more than the colour of the fluorescence. F. Perrin [90] has investigated this, and has suggested the exponential relationship $\phi = \phi_0 e^{-kc}$, where ϕ_0 and ϕ are the initial and observed intensities of fluorescence, respectively, and c is the corresponding change in concentration of the fluorescent substance, k being a constant characteristic of the latter. The importance of diffusion is indicated by the fact that (as A. A. Dixon has shown [92]) in the case of solid solutions k is about 2·9 times larger than in liquids. S. J. Wawilow [93] found that, for very low values of c, ϕ attains a constant value. Table 4 shows the concentrations at

TABLE 4.

Substance.	Limiting Concentration. One Part in	Solvent.
Æsculin	10^{10}	Water
Bilirubin	12×10^6	Alcohol
Fraxin	10^8	Water
Hæmatoporphyrin	10^9	$N.H_2SO_4$ or $N.NH_4OH$
Hydrastinine	5×10^6	Alcohol
	2×10^7	HCl
	10^{11}	H_2SO_4
Quinidine	2×10^7	H_2SO_4
Quinine	$0·5 \times 10^8$	H_2SO_4 [94]
Urobilin	10^6	Water

which fluorescence is visible for a number of organic substances; it indicates the sensitiveness of the method, but as the figures are compiled from the results of different workers, differences in working conditions (*e.g.*, in methods of irradiation and observation) must be taken into account. The colours are all shades of blue except those of bilirubin and hæmatoporphyrin (red) and urobilin (green). Perrin's relationship ceases to hold at high concentrations.[134]

Temperature.—Although in general ultra-violet fluorescence may be quenched by irradiation with infra-red (*i.e.* heat) rays, and certain substances (*e.g.*, glycerol, sulphuric acid and dextrose) have a weaker fluorescence at high temperatures, cases are known (*e.g.*, æsculin [77]) where the reverse applies. Kautsky, Hirsch and Davidshöfer [76] found that the duration of the fluorescence of substances cooled in liquid air was trebled, a spectral shift towards the region of longer wave-length being also produced, and the former is attributed to the minimisation of deactivation when the molecular velocity is low (see also Lewschin [75]). J. Bouchard [140] states that Perrin's law holds between 0° and 80° C.

H. Eichler (see p. 293) has made the interesting observation that solid solutions of Magdala Red in certain compounds having phenolic or carboxylic groups are violet and non-fluorescent in the solid state, although at the melting-point a strong yellow-red fluorescence develops. He proposes to use this property to operate a fluorescence thermoscope. For temperatures below 0° C. suitable solvents are methyl and ethyl alcohols, glycerol and acetone. For higher temperatures thymol, phenol, *o*-, *m*-, and *p*-cresols, benzoic, salicylic, *o*-phthalic, acetic and formic acids are suggested as solvents, as with these the change from the inert to the fluorescent state at the melting-point is very sharp.

Ageing and Fading.—The change in the colour or intensity of the fluorescence which frequently occurs while a substance is being irradiated may lead to false results if it passes unobserved. It is of special importance in connection with capillary analysis (p. 58), and with fading tests (p. 344), and is discussed more fully nnder these headings. Oxidation, both purely photochemical and as a result of dissolved oxygen or of ozone produced by the lamp, has been suggested as a possible explanation in the case of dyes (see J. Becker,[85] D. N. Chakravarti and N. R. Dhar,[86] and K. G. Mathur and S. S. Bhatagnar [87]), and numerous other

instances and explanations occur in the literature, also usually in connection with dyestuffs.[79-84] Haitinger[60] has illustrated the effect of dilution on the rate of change of the intensity of the fluorescence of æsculin; thus, after 10 minutes of irradiation a 0·002 per cent. solution was unaffected, whilst the fluorescence of a 0·0006 per cent. solution decreased in intensity by 5·5 per cent.

M. Haitinger and V. Reich[7] have investigated the change of the colour of the fluorescence for a number of substances in the form of fine powders or on filter-papers, and the change in the fluorescence of milk on ageing is mentioned by O. Gerngross and M. Schulz (see p. 155). Solutions of chlorophyll exposed to the unfiltered light from the lamp cease to fluoresce after some time, this change being slower in the filtered light. The phenomenon is observed with æsculin in dilute aqueous solution, but here the change continues even when the solution is placed in the dark.[8] According to P. Wels,[9] serum globin in filtered ultra-violet light fluoresces with a blue colour, and after a period of exposure to the unfiltered light the intensity of the fluorescence is found to have increased. J. Becker[10] mentions the change in the colour of the fluorescence of certain oils and peptone solutions, while J. Lenfeld and others have shown that exposure of butter to air and light for some time produces a change in the fluorescence colour (see p. 151). L. H. Lampitt and co-workers[106] have described a method for irradiating fats, the intensity of the ultra-violet light being measured by means of a photo-electric cell.

Impurities.—The fact that the purity of the substance under examination can in certain cases have an important effect on the nature and intensity of the fluorescence, is one of the weaknesses (and also of course one of the advantages) of fluorescence analysis. Examples will be found in all the Chapters (see especially IX and XIII), and the matter is considered from the point of view of inhibition and activation in inorganic chemistry on page 202; the nature of the solvent may also determine the nature and extent of inhibition (*vide supra*). Minerals (see E. H. Kranck and others[88]), and luminous paints also provide some interesting examples, *e.g.*, artificial willemite fluoresces only if manganese is present, and zinc sulphide requires the presence of copper. It has even been stated (by A. Petrikaln[89]) that the fluorescence of

intensively-dried substances is diminished, quinine sulphate being cited as an example.

The general question of the influence of foreign substances on the fluorescence of solutions is becoming one of some considerable theoretical importance, although it has only a slight bearing on fluorescence analysis. J. Bouchard [141] has shown, for example, that the addition of electrolytes which increase the degree of molecular association of the solute decreases the fluorescence, and that the most active inhibitors are antioxidants. The relationship of inhibiting power to oxidation-reduction effects is further demonstrated by the fact that, as H. Hellström [142] and K. Weber [143] have shown, the fluorescence of methylene blue is inhibited by the addition of ferrous salts, and to an extent which depends on the *p*H value. A. Boutaric, J. Bouchard and C. Achard [144] have shown too, that certain alkaloids inhibit the fluorescence of uranine solutions, but that the corresponding genalkaloids (formed by converting the amino-group into an amino oxide-group) do not; the genalkaloids have therapeutic properties which are similar to those of the alkaloids, but they are less toxic. J. Weiss and H. Fischgold [146] ascribe the phenomenon of quenching to the transfer of an electron from the quenching ion to the excited moelcule. K. Weber,[145] on the other hand, failed to establish that oxidation-reduction potentials are a measure of the degree of quenching of the fluorescence. Examples illustrating quenching effects occur elsewhere in this book, notably in Chapters VIII and XIV.

Estimations of Mixtures.—The percentage of fluorescent substance in admixture with a non-fluorescent substance may often be estimated. F. Hein and W. Retter [4] have investigated microscopically mixtures of zinc oxide, uranyl sulphate and anisil with various non-fluorescing substances such as copper oxide, red lead, chromium oxide, iron oxide and cinnabar. A mixture of 1 part of zinc oxide with 10 parts of cinnabar showed a distinct fluorescence, and when the mixture contained 100 parts of cinnabar, a greenish-yellow fluorescence was still detectable in intimate mixtures. Further instances will be found in other chapters (*e.g.*, on p. 194) and below (Hirst [78]).

Preliminary Qualitative Examination.

A good general outline of the course to be pursued in a preliminary examination of a sample under the lamp is given by Guyot,[5] and in this connection it should be emphasised that sufficient time (*e.g.*, 5 minutes) should always be allowed for the eyes to acclimatise themselves to the dark surroundings, in order that the maximum effect of the fluorescence may be appreciated. This will also allow for any initial variations in the output of the lamp, and for the ozone which is produced at this stage and which has been stated to have a bleaching effect in certain cases.

Solids.—If the surface is fairly large it may be examined directly in the rays, and at a later stage, photographs taken in daylight and in ultra-violet light may be compared. The sample should then be broken and the fracture examined under the lamp to see if air and light influence the fluorescence, and if so, observations should be made on freshly-broken surfaces. The surface should then be spotted with (*a*) a dilute acid, (*b*) alkali, and re-examined.

Powders.—The sample may be examined directly and then shaken up with a non-fluorescent liquid and re-examined. Very fine powders may be blown on to a damp, non-fluorescent filter-paper for examination, but it is best to dry the paper before examination, as moisture has been shown to change the fluorescence colour of certain fine white powders (see p. 194). The action of heat on the fluorescence of the powder may also be noted.

Liquids.—A number of methods have been proposed as suitable for routine examination. The sample should be placed in a non-fluorescent (*e.g.*, quartz) tube and examined under the lamp, and it should be agitated during the examination. The fluorescence, both at the surface and in the body of the liquid, should be noted, and also the fluorescence of the condensed liquid when the sample is boiled. A drop may also be held between two quartz microscope slides and examined by reflected or transmitted ultra-violet light. It is often a good plan to dilute the liquid with another, non-fluorescent, liquid and to examine the mixture at progressively increasing dilutions, as a change in the intensity or colour of the fluorescence sometimes appears

under these conditions, and also, there is often an optimum dilution for which the fluorescence has a maximum intensity. The tube may be tilted so as to cover the walls with some of the liquid, and this helps to eliminate other colour effects.

Colorimeters (see also p. 63).—In order to observe the colour of the fluorescence, Lundén [3] used a frame (Fig. 11) containing coloured glasses for the examination of the fluorescence of sugars, and a set of accurately-graded coloured glasses allowing only rays of specific colour regions or wave-length ranges to pass, would be very useful if it could be produced at a low cost.

M. Haitinger and V. Reich [6, 7] have studied this problem and have designed an apparatus to eliminate some of the difficulties. The most favourable conditions are those in which the ultra-violet light falls directly on the surface of the liquid without touching the external walls of the vessel. The tubes are therefore supported by wire clips in an upright position and are separated by vertical dividing walls. Between the tubes and the glass filter is placed a piece of wood or cardboard having circular openings about 20 mm. in diameter, and in each opening is inserted a piece of tube about 25 mm. long. The ultra-violet light can therefore only enter the test-tubes through the vertical tubes in the board, the whole apparatus being supported so that the partition is horizontal and the tubes are vertical.

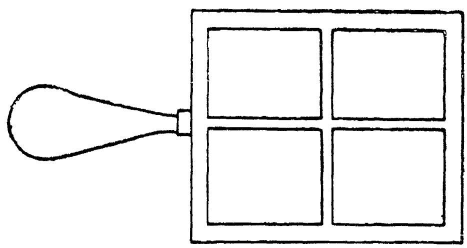


FIG. 11.

With a 150 mm. filter three such tubes may be conveniently examined at one time, and comparable results may thus be obtained (see Fig. 12). All cardboard and wooden portions of the apparatus should be covered with black matte paper, and care should be taken to ensure that no stray light passes into the tubes from the back and sides of the lamp-holder. If photographs are to be taken, all surfaces except those of the tubes should be covered with black velvet so that there may be no reflection of light to interfere with the exposure.

In this method only the true fluorescence colour of the liquid

is observed; thus, with liquid paraffin a sharply-defined yellow-green ring is seen, instead of a dense blue throughout the whole contents of the tube. The method also enables the concentration of the liquid to be estimated from the depth to which the fluorescence penetrates. With very concentrated solutions only a very narrow ring is illuminated, whilst on progressive dilution the fluorescence penetrates further and further, being sometimes accompanied by a colour change, until finally a degree of dilution is reached at which the fluorescence is visible down all the length of the tube. The method described by H. R. Hirst,[78] in which the fluorescence of oils is matched by comparison with mixtures of

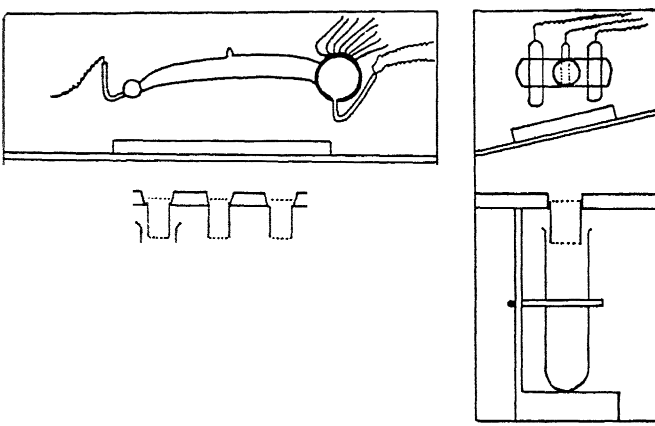


FIG. 12.

solutions of quinine sulphate (blue) and Indigosol-O (green) could probably also be applied and extended in this connection.

Capillary Analysis.[51]—Another method for the examination of liquids, especially solutions, is that due to P. W. Danckwortt and E. Pfau, and it has been applied with great success to the examinations of solutions of drugs (see p. 134), resins,[62] etc. If a filter-paper is held vertically with one edge in the solution, it draws up the liquid by capillary attraction, and when the wet portion is examined under the lamp, characteristic zones are obtained from many substances. The test does not give comparable results unless a standard procedure is followed.

The paper should be non-fluorescent and should be cut into strips 2 by 30 cm., all strips being cut in the same direction,

preferably along the wire- or machine-direction of the sheet, since the absorption varies according to the grain of the paper. The paper is then clamped by its upper edge so that it hangs vertically, 5 mm. of the lower edge being immersed in the solution, and it may be left for so long as 24 hours in order to ensure that a state of equilibrium has been reached; temperature, concentration, humidity, air currents and time must all be carefully controlled if comparable results are desired. The papers obtained are examined under the lamp, and the results are compared with

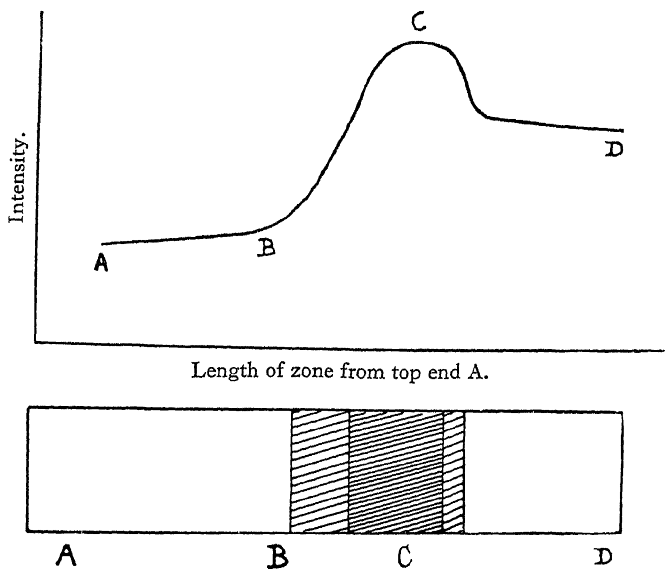


FIG. 13.

those obtained from samples of known compositions and concentrations.

If in addition to the colour, length and spacing of the various zones, their intensities are also measured by means of a photometer, the results obtained may be plotted as shown in Fig. 13.

Fig. 13 also shows a diagrammatic representation of the actual strip of filter-paper. It is then found that a characteristic curve is obtained for each substance, the intensely coloured zones (B and C) being represented by corresponding positions marked on the curve; M. Guyot [59] was thus able to obtain quantitative results. If the sample is an oil, a solution in ether is used.

It is obvious that any solvents used in this work should be optically inert and non-fluorescent, and ether, petroleum spirit, amyl alcohol and chloroform are widely employed. The choice and purity of the solvent are often important, and, as indicated on page 290, a whole range of colours may be obtained from the same substance dissolved in a number of different solvents. In aqueous solutions the *p*H value of the liquid medium is often of great importance, and the fluorescent indicators used for alkalimetry, acidimetry and general volumetric analysis (see p. 310) depend on its influence.

Extraction of a liquid with one of the solvents mentioned above is often of assistance in this work, and adsorption on substances such as kaolin, tristearin, silica gel, aluminium hydroxide or cotton wool is also sometimes used.[61] Numerous examples of the use of extraction or adsorption will be found in subsequent chapters. W. Grassmann and O. Lang [101] used tubes containing aluminium oxide or magnesium oxide to obtain chromatograms of various tanning agents. Their method is easy to carry out, and the results, although dependent on the *p*H value of the solution, the medium used and the nature of the tanning agent under examination, are readily reproducible and characteristic of the substance examined. The bands or zones so produced are stable for from 1 to 2 days.

To a 20 to 30 per cent. aqueous extract of the tanning agent is added an equal volume of methyl alcohol, and after centrifuging, the clear liquid is poured into the top of a quartz tube packed with the adsorption medium ; or the tube may be placed vertically in the liquid, which is allowed to rise by capillary action. After some time the tube shows under the lamp a series of fluorescent zones, which can be further extended and parted by washing the medium with ethyl acetate, methyl acetate or ligroin. The method appears capable of wide extension, as by its use the components responsible for the fluorescence of tanning extracts could be distinguished. The adsorption medium should be damped with the aqueous alcohol first and other variations in procedure lead to the production of different results, but when a worker has found a technique suitable for his purpose his results are reproducible and reliable.

This method has also been used by F. Bandow [102] to examine the position of the maximum band in the fluorescence spectra

of the porphyrins dissolved in acid, neutral or alkaline organic solvents. He used aluminium oxide, chalk, franconite, floridin and kaolin, and found good correspondence between the fluorescence spectra in the adsorbed state and in solution.

E. R. Bolton and K. A. Williams [103] give a good description of the chromatographic analysis of oils and fats, and their work suggests the use of this and fluorescence analysis for the detection of fuel oil in whale oil. H. Thaler,[104] using benzene solutions, aluminium oxide and a Bavarian clay (Clarit) as adsorbents, has obtained chromatograms of the colouring matters in butter. He finds that synthetic dyestuffs, and even artificial carotenoids, are more strongly adsorbed than natural carotenoids, but considers that, in this case, the use of ultra-violet light offers no advantage over daylight.

It is sometimes desirable to coat an apparatus or part of an apparatus with a material which reflects ultra-violet light. According to M. Luckiesh [11] (who has collected many data on reflection) and L. L. Holladay,[12] a reflecting-power of 50 to 65 per cent. is obtainable from a mixture of magnesium oxide, magnesium carbonate and aluminium oxide containing small amounts of silica, chalk and kaolin; the binding medium may be either casein or a nitrocellulose lacquer.

Quantitative.

With the possible exception of capillary analysis, which is semi-quantitative, the above methods are mainly qualitative, and the more quantitative side of this work now requires attention. In direct visual observations the personal factor is an important consideration, and an examination of the various published papers reveals a wealth of verbal licence in the matter of descriptions of the colour of the fluorescence; terms such as "white with a blue tinge" and "pale bluish-white," for example, are apt to lead to some confusion, and although the difference between such colours may be quite apparent to the observer, the terminology is somewhat unscientific. It will be appreciated that the eye is a synthetical and not an analytical organ. Hence a colour seen and described as blue may be shown to have a red band when examined spectroscopically, and quite a striking case is that of the yellow tones which may appear yellow to the eye and yet be made up of red, blue and green components. With

many yellow screens or filters a considerable proportion of the near ultra-violet region is transmitted, so that in fluorescence analysis it is preferable to analyse the resultant radiation in order to obtain strictly comparable results.

Spectroscopy.—W. Scheffer,[13] Bayle and Fabre,[14] and other workers have used the spectroscope for the quantitative examination of the brightness and colour-components of fluorescence colours (see especially C. Dhéré [91]). A. Policard [15] used a fluorescence microscope (see p. 78) in conjunction with a spectrophotometer, and between the lamp and the spectroscope he placed a polarisation apparatus and so was able to obtain the desired spectrum and also a comparison spectrum of equal intensity. The colour and the corresponding intensity of the fluorescence may then be expressed in the form of a graph in which the wave-lengths of the light are plotted as abscissæ and their intensities as ordinates.

Bayle [16, 17] followed the intensity of the fluorescence on dilution of a solution by means of a spectrophotometer. From the resulting curves it appears that for many substances there is an optimum concentration for which the fluorescence is a maximum, a fact already mentioned on page 52. For quinine in sulphuric acid this concentration is 1 : 500 and for hydrastine it is 1 : 2500, at 5300 Å. in both cases. According to F. Perrin [18, 19] the fluorescence is an exponential function of the concentration, and the relation between intensity, concentration, viscosity and temperature is treated briefly on page 381.

These instruments are all fairly easy to use and, in particular, the spectroscope is of such great value that a few further details concerning it are desirable. W. Scheffer,[13] for instance, recommends that the substance to be examined should be placed in front of the slit of the instrument, and, by means of a quartz lens or other suitable device, the ultra-violet rays may then be concentrated on it. A bright point of light results, and this can be examined in the spectroscope in the usual way. If a photograph of the fluorescence-spectrum is required, one of the screens mentioned on page 70 should be interposed between the slit and the sample in order to cut out any reflected ultra-violet light. The spectrum of the source of light should also be determined before the sample is brought into position since, owing to reflection, lines from it may otherwise appear in the spectrogram.

The work of Bayle and Fabre and co-workers [16, 17] is im-

portant, since they used a *spectrophotometer* for the measurement of the wave-lengths of the fluorescence of a number of organic compounds and the corresponding intensities, and they plotted the results obtained graphically. They obtained characteristic curves for a number of substances, the maxima of which were appreciably altered in position by the presence of impurities. Thus, with hydrastine in the crude state, a yellow-green fluorescence is obtained, but the corresponding curve is modified after recrystallisation several times from benzene. In this way the purification of certain organic substances may be followed and controlled (*cf.* also J. Wawilow [119]).

For the estimation of quinine in blood (*cf.* p. 313) F. J. Kaiser [107] compares the fluorescence of the solution under examination with the light transmitted by a cell illuminated by a 6 volt, 40 watt lamp mounted on an optical bench. The cell contains a solution of Patent Green and Pyrrole Blue, which matches the colour of the fluorescence of quinine sulphate. The lamp is moved until the match is obtained, and the distance on the optical bench between the lamp and cell is noted. Thirty standard solutions containing amounts of quinine sulphate ranging from 8·0 to 0·5 γ of quinine are made up, and two are selected for comparison, one having a slightly greater, and the other a slightly less intensity of fluorescence than that of the sample. These are in turn matched, and the distances between the cell and the lamp is noted. Now if the standard contains $s\gamma$ of quinine and the optical bench reading is a cms.; and if for the standards containing y and $(y + 0{\cdot}25)$ γ of quinine the readings are b and c cms., respectively, then $c^2/a^2 = s(y + 0{\cdot}25)$, and $b^2/a^2 = s/y$. Two values of s may thus be calculated and the mean value obtained. Kaiser finds the mean error by this method to be 4 per cent., and the minimum amount of quinine that can be determined to be of the order of 0·5 γ.

Colorimeters (see also p. 57).—L. J. Desha [20] has employed a Kober *nephelometer* for the study of fluorescence, a similar type of instrument being used by P. Wels,[9, 124, 125] and by H. Wüstenfeld and H. Kreipe and others [120-123] in their work on the fluorescence of vinegars (see p. 170). A colorimeter of the Duboscq type was used by A. Mangini and R. Stratta, and the *step-photometer* has been found useful for fat analysis by W. Sproesser and by H. P. Kauffmann (see p. 155). The

Lovibond tintometer has also been used with success by Cocking and Crews (see p. 142), and other colorimeters by A. and W. Thiel[117] and by K. Weber.[118]

Morgan and MacLennan[21] (see p. 149) have examined the fluorescence of butters and margarines by means of the *Guild colorimeter*, and their apparatus is worthy of detailed description since it was one of the first to give strictly comparable results

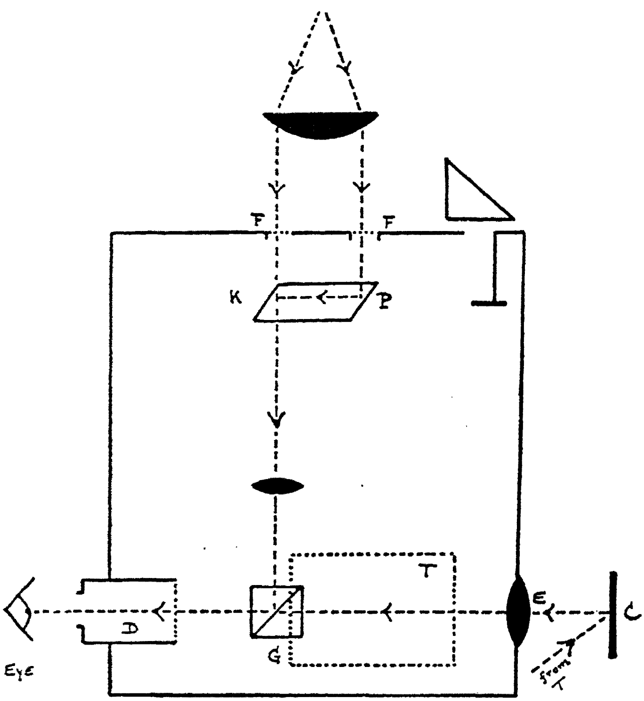


FIG. 14A.—(Plan.)

with a variety of substances. Figs. 14A and 14B indicate the salient features of the apparatus. The colour of the fluorescence of the sample is observed through the eyepiece and covers half the field of view, the other half of which is occupied by a colour made by combining the three primaries, red, blue and green. The amount of each colour is controlled by adjusting the aperture of the three respective colour filters and, by superposition of

A. P. H. Trivelli, *Sci. Monthly*, 1931, **33**, 175; (with L. V. Forster) *Jour. Opt. Soc. America*, 1931, **21**, 124 (Technique of fluorescence microscopy).

K. Reichert, *Optik*, 1932, Nos. 8-9; H. Heine, *Zeit. Wiss. Mikros.*, 1932, **48**, 450 (Fluorescence microscopy).

A. Grabner, *Phot. Korrespond.*, 1933, **69**, No. 5 (Colour fluorescence photomicroscopy).

L. Vinokurov and V. Levshin, *Compt. rend. Acad. Sci. U.R.S.S.*, 1936, **2**, 135 (Extinction of fluorescence).

K. C. Bailey, "The Retardation of Chemical Reactions" (London: Arnold), 1937 (Quenching of fluorescence).

W. Szymanowski, *Bull. Akad. Polonaise*, 1925, **A**, 34; *Zeit. Phys.*, 1935, **95**, 440, 460, 466 (Influence of electrolytes, concentration and the viscosity of the solvent on the rate of decay of the fluorescence of uranine).

L. M. Ananèva and A. A. Shishlovskiĭ, *Compt. rend. U.R.S.S.*, 1937, **17**, 183 (Sensitisation of photographic plates by means of sodium salicylate).

G (green), and B (blue), until the required shade is obtained, and the field as viewed through the eyepiece appears uniform. The aperture-readings of the colour filters are then read, and provide a numerical representation of the analysis of the fluorescence colour. Thus, for example, the colour value of a certain yellow fluorescence is given by the figures "red 27, green 44, blue 29," whilst a buff colour is "red 53, green 31, blue 16."

In the *Donaldson Colorimeter* the light is mixed by passing it into a sphere having a highly-reflective inside surface; it is otherwise similar in general principle to the Guild colorimeter.

Another comparison method described by Haitinger[22, 60, 74] gives a quantitative expression of the colour and intensity of fluorescence, a *Zeiss step-photometer* being used to obtain a match between the sample and the standard (barium white) as seen through red, green and blue filters. The numerical values obtained in each case are plotted as percentages of the total light emitted, in a Helmholtz-König triangular colour diagram. Thus, for example, since white contains equal proportions of the three constituent-primary colours, the point representing it would therefore be placed equidistant from the three apexes of the triangle. The tone, degree of saturation and intensity of the fluorescence of a particular substance may thence be assessed quantitatively since the shade of the fluorescence colour is given in terms of two rectangular co-ordinates.

A variation of the same principle is described by W. Buchloh,[66] who interpreted his readings with the Pulfrich step-photometer by means of the Ostwald cone-colour theory, in which two cones having a common base, and apexes representing black and white, respectively, are used to picture the relationship of the primary colours and their mixtures which are placed on the circumference of the common base.

L. J. Desha[20] points out that the light emitted by a fluorescing substance in solution is a function not only of the concentration of the substance, but also of the light absorbed. If all the incident ultra-violet light is absorbed at one concentration, an increase in the concentration will make very little difference, if any, to the fluorescence. Thus it is important, when using a nephelometer or other instrument to measure the concentration of a substance in solution, to work at a suitable dilution, *e.g.*

by diluting the sample until further dilution produces a marked diminution in fluorescence; better still, a cell containing the solution is interposed between the lamp and a vessel containing a portion of the solution, and the liquid in one cell is diluted until the liquid in the other commences to fluoresce. At this stage all the ultra-violet light is not being absorbed in the first solution, and hence a further change in concentration will influence the intensity of its fluorescence. The *p*H value of the two portions should be the same.

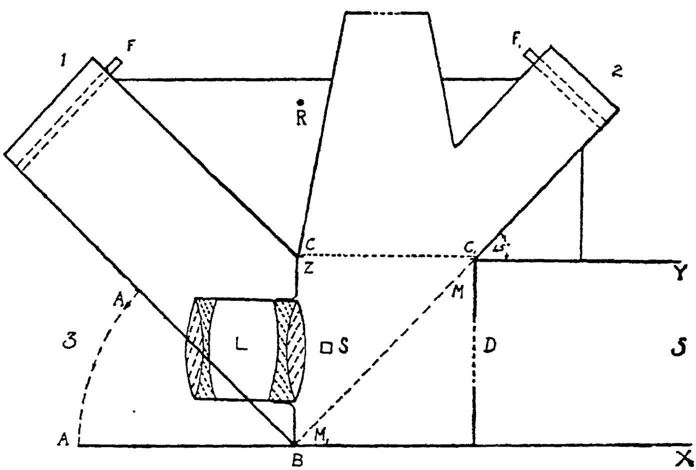


FIG. 15.

A photometer in conjunction with a photo-electric cell for measuring fluorescence has been employed by W. West [115] and co-workers, and by R. Toussaint.[116].

An ingenious piece of apparatus introduced by S. Mohorovičič [23] fulfils the functions of an observation chamber for fluorescence and a nephelometer, as well as of a photometer and projection apparatus. Its principal features are several tubes for viewing the object under examination or for irradiating it. The object may also be photographed through one tube, viewed through another and irradiated through a third, simultaneously. A general idea of the construction and use may be gathered from Fig. 15.

BXYZ is a laterally-removable door which gives access to the mirror MM_1 and the screen CC_1 both of which may be removed. AB shows another door in the side of the tube 1 which may be opened so as to allow a system of lenses L to be inserted. FF_1 are slits for the purpose of inserting filters (10 × 14 cm.) either for ultra-violet or for monochromatic light; two are shown in position. The opening at the end of the tube 4 can be closed, and a camera, spectroscope or other apparatus may be attached at XY. The fluorescent body is placed at S and can be irradiated by light passing through tubes 1, 2 or 3. The whole apparatus may be rotated about the horizontal axis R, in the vertical plane, and a vertical circular scale is also included.

A. Thiel [108] conducts the fluorescent light through a glass rod, the end of which forms half the field of vision in the eye-piece of the instrument, the other half being the end of another glass rod which is itself fluorescent, the colour of this fluorescence being modified by inserting colour filters. Photometric methods for measuring the fluorescence of X-ray screens have been described by H. Funk and H. Steps,[109, 110] and the use of photo-electric cells by F. H. Cohen,[111] and others.[112]

Another type of instrument sometimes used is known as the *Fluorometer*, its function being to measure the duration of fluorescence. It is based on the principle of Becquerel's phosphoroscope, in which the fluorescent body is irradiated successively through a series of holes in one disc, and viewed through a series of holes in another similar disc. Both discs can be rotated at high speeds, and are so geared together that the object is never viewed at the same time as it is irradiated. The exciting light passes through a slot and falls on the object; the rotation of one disc then shuts off the light, and an aperture in the second disc passes in front of the body allowing it to be viewed. This process takes place in rapid alternation, which is controlled by the speed at which the discs rotate. When, through the viewer the fluorescent light is practically extinguished, it is possible to calculate from the speed of rotation and disposition of the slots in the two discs the time elapsing between the irradiation and final extinction of the fluorescence. Various types of fluorometers have been described by F. Duschinsky,[113] and in one instrument (O. Maercks [114]) the exciting radiation is interrupted by a train of supersonic waves of known frequency

and the fluorescent light interrupted by the same or another train of supersonic waves, the resulting displacement being a measure of the duration of the fluorescence. With this instrument Maercks claims an accuracy of 2×10^{-10} second. Numerous other methods are cited in the references.[67-74, 126-128, 150]

Photographic Methods.

Photography enters largely into many of the methods mentioned above, since it is important to be able to reproduce images of fluorescence phenomena, particularly as a means of demonstrating and clarifying results. The term "luminogram" has been applied to such photographs by W. R. Mansfield, and a number of these are reproduced facing page 400 *et seq.*

There is often a great difference between photographs of an object taken in daylight and in ultra-violet light, and as indicated on page 227 it is often important for the legal chemist to be able to present in court a photograph illustrating a certain point, which may be circulated among the jury. Kögel in fact considers that when the result of a case depends on evidence obtained by means of the lamp, a photograph is essential if the evidence is to carry any weight. Photographic methods must, however, always suffer from the handicap that they are unable to reproduce colour-effects faithfully, and in the case of fluorescence phenomena (which are characterised by very subtle differences in colour) this is a particularly adverse criticism. H. W. Meyer,[136] however, has recently shown that good results can be obtained by the use of the tri-colour films now available commercially.

It is necessary to distinguish clearly at this point between two types of such photography. In the first, the *fluorescent light* emitted by the object, and in the second the *ultra-violet light reflected* from the irradiated object is all that is recorded on the plate (see Frontispiece). The two types are conveniently known as *Fluorescence photographs* and *Ultra-violet light photographs*, respectively.

Fluorescence Photographs.—H. Miethe,[24] who has published some very fine photographs of various fluorescence phenomena (see Photograph No. 5, facing p. 400), points out that the difficulties attending photographic reproduction of these effects appear, at first sight, to be considerable. In the first place, the

ordinary plate is more sensitive to rays in the ultra-violet region than in the visible region, and moreover, the rays affecting the plate are made up of the reflected ultra-violet rays and the comparatively feeble visible rays of the fluorescence. The problem then, was to find a filter capable of cutting out the ultra-violet rays without diminishing the intensity of the visible light.

As mentioned in Chapter III (on filters), Miethe used a cell containing a filtered 1 per cent. solution of cerium ammonium nitrate which cuts out the ultra-violet rays of longest wave-lengths. Vodrážka [99] immersed an unexposed photographic plate in a 10 per cent. solution of this salt for 15 minutes; it then cut off rays below 4000 Å. G. Kögel [25, 26] prefers a solution of 0·5 grm. of triphenylmethane in 70 c.c. of absolute alcohol, but in this case care must be exercised to ensure that the cell is completely closed, as otherwise moisture from the air is absorbed by the alcohol and the filter is thereby rendered useless. A solution of didymium nitrate in water is recommended by W. Scheffer,[27] and according to K. Weber [28] a 2 per cent. solution of sodium nitrite in water gives excellent results in thicknesses of 1 cm. Other filters recommended are Wratten No. 2 A,[138] Corning Noviol C, or Wratten K2.[139] A disadvantage of the use of solutions as filters is their lack of permanence, and this applies particularly to cerium ammonium nitrate solution, which should be freshly-prepared each time it is required.

The apparatus used by Kögel is somewhat too expensive and cumbersome for the average worker. A ray of polychromatic light is passed in succession through a condenser, a slit and two prisms, the final spectrum being from 1 to 2 m. wide and about 0·5 m. in height. Naturally, this large spectrum gives one considerable advantage in the choice of the ultra-violet spectral-regions to be used in photographic work. As a result of the work of Judd Lewis (p. 341), it is apparent that if a photograph is taken of an object irradiated first with light of one and then of another range of wave-lengths it is possible to obtain several negatives differing in density according to the intensity of the emitted light. Kögel recommends the line at 3130 Å. as most widely applicable to this branch of work, and this line appears in the spectrograms of certain commercial filters, such as the Hanau and the 5 mm. red-purple Corex filter, and in Corex A, 986 A, of the Corning Glass Co.

G. C. Brock [133] has introduced a simple method of obtaining a filter which gives a strong band at 3130 Å. and which is superior to the thin silver film-filter sometimes employed (see also p. 34). A lantern plate, such as the Wellington "SCP," Paget Gravura, and Criterion "Mezzochrome," is exposed to even illumination and is then developed to a warm brown-red tone. In the case of a "Gravura" plate exposure is for 2 minutes at a distance of 8 inches from a 60-watt "Pearl" bulb, and it is developed for 7 minutes at 70° F. in a mixture of 15 c.c. each of sodium carbonate and pyro solutions, and 10 c.c. of a 10 per cent. solution of potassium bromide. The film is then stripped from the plate using Steery's method (B. J. Almanac [149]); it is washed free from any greasy scum, and after drying is clipped between two plates of quartz or vita-glass. Care should be taken at the stripping stage not to damage the film, and it should be borne in mind that the additional wetting and drying of the film when removing the greasy scum increases the density of the negative, so that this should be allowed for in the preliminary stages.

In the case of this filter it is probable that the filter action is due to the presence of colloidal silver of a certain density and particle-size, as plates having a reddish-brown colour are found to be the most suitable.

To photograph fluorescence two filters are necessary, *viz*., one in front of the lamp so that only ultra-violet light passes to the object; and one in front of the camera to absorb any of this ultra-violet light which may be reflected, although it must transmit the visible light of the fluorescence.

For ordinary routine work a simple apparatus (Fig. 16) can be easily set up and left as a permanent fixture in the dark-room. The camera C, which may be any type of view-camera, stands from 75 to 100 cms. from the object clip, and its lens need not be of quartz (since we are not recording ultra-violet light). A glass-walled filter-cell containing one of the solutions mentioned above is placed in front of the camera at F, B being an optical bench and LL sources of filtered ultra-violet light. If the object (S) has a flat surface, as in the case of textiles and papers, oblique illumination is best, and if two lamps are available, as assumed in Fig. 16, one on each side will ensure uniform illumination and a negative free from shadows. If the object has a highly-reflecting surface, such as porcelain, glass or marble, etc., the lamp

is placed at an angle of 45° to the line between the object and camera (CS) so that very little ultra-violet light is reflected to the camera.

Fig. 17 (by courtesy of Mr. H. R. Hirst) shows a similar arrangement with a single lamp. The source of ultra-violet light is a mercury arc of the Kelvin, Bottomley and Baird "Gallois" A.C. type, with an arc-tube voltage of approximately 150 volts, and the current passing through the mid-point of burner is about 5 amperes. The camera is placed on a wooden frame so arranged that the object is square with the plate, and the lens is $12\frac{1}{2}$ inches from the object using a

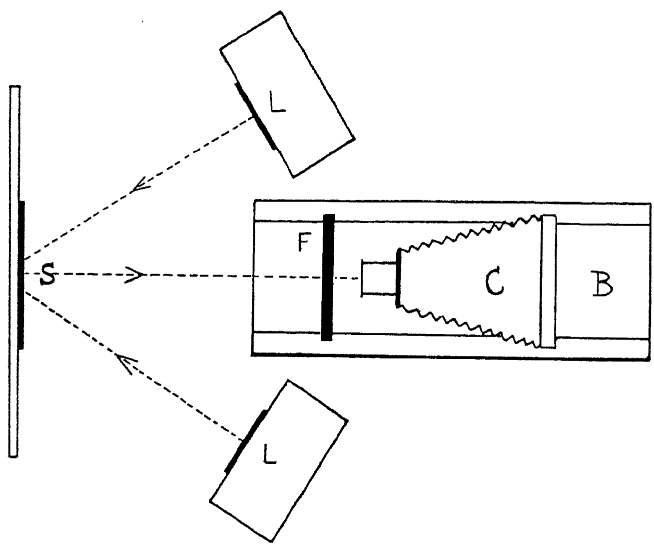


FIG. 16.

10·5 mm. focus lens. The light is filtered with a Chance's glass screen which is 19 inches from the object. It is necessary to exclude ordinary light and to enclose the camera and lamp in a wire frame covered with black cloth.

The lamp should be allowed to run for 10 to 15 minutes, when it should be burning steadily and with its maximum output. When large surfaces (*e.g.*, oil paintings) are to be photographed, the filter F in Fig. 16 is placed practically touching the camera lens. In such cases a large filter may be constructed from a number of small filters set edge to edge, one above the other,

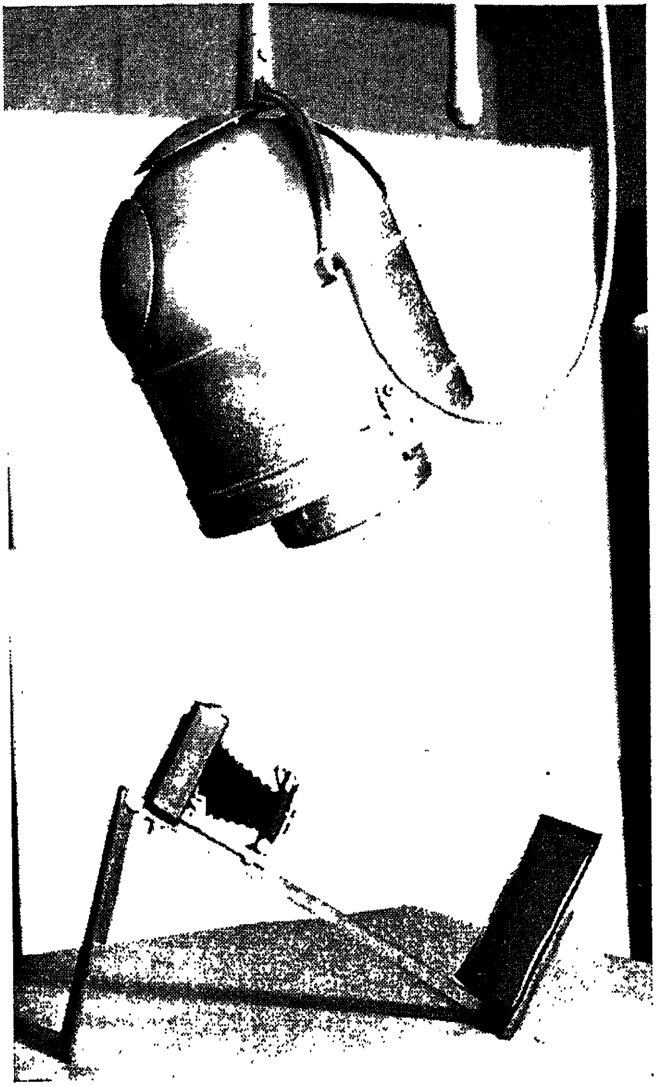


FIG. 17.

[*To face page* 72.

and a large cell may easily be constructed when a liquid filter is required ; J. A. Radley has used a small cell, nearly touching the lens, for this purpose.

A novel source of ultra-violet radiation which is stated to be specially suitable for the photography of documents is due to L. Bendikson.[96,137] The source is a sealed, quartz, spiral discharge-tube, encircling the lens and containing a mixture of helium, argon and mercury vapour at a low pressure. Behind it is a metal reflector, through which projects a cylinder which acts as a hood to protect the lens, and which is also designed to carry filters cut to fit round the latter. It is claimed that this arrangement is cheap ; requires short exposures ; provides a form of direct surface illumination which minimises shadows due to wrinkles and folds ; minimises heat radiations and so avoids the danger of injury to the object. About 70 per cent. of the radiation is between 2540 and 2570 Å., and about 18 per cent. has a wave-length of about 2900 Å. This lamp may be used as a ring-illuminator, and it has also been adapted for rapid successive photography in visible and in ultra-violet light.

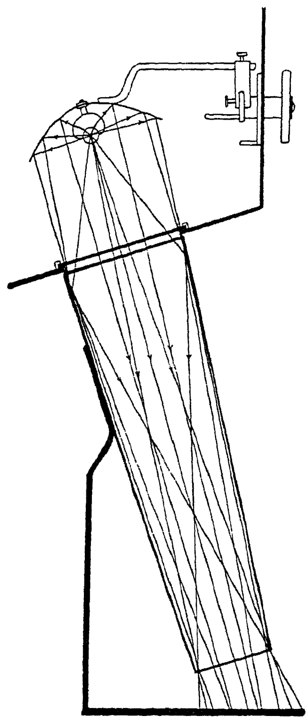


FIG. 18.

Concentrators. — For photographs of small objects which are to be enlarged, the rays should be concentrated by passing them through a lens made of quartz or through a thin glass cell, with good powers of transmission for ultra-violet light, filled with water ; both the glass and the water should be free from dust. Several other concentrating devices have been suggested,[29] chiefly for microscopical work, *e.g.*, J. Grant has used a spherical flask containing distilled water as a lens. Tiede and Goldschmidt suggest a small lamp placed at the focus of a parabolic reflector, so that the rays pass through a

lens and then down a funnel at the end of which is placed the object to be examined (Fig. 18).

G. Kögel [30] used a chromium-plated reflector instead of a quartz optical system, and an ordinary concave microscope lens also gives satisfactory results.

Plates and Exposure.—J. J. Rorimer [44] (see also p. 286) considers that it is not possible to record on the photographic plate certain fluorescence effects when the Corning glass filter "Noviol O" is used, as this cuts out the most active actinic rays (*i.e.* shorter than 3669 Å.). He concludes that ultra-violet photographs made with an anastigmatic lens are largely dependent on the action on the plate of rays of about 3669, 3660 and 3633 Å.

One of us (J. A. R.) with N. J. Gruber has obtained photographs which show the distinct variations in the fluorescence of milks and papers (*cf.* p. 77) on the panchromatic plates of several reputable firms, by means of a cerium ammonium nitrate lens filter and the ordinary Hanau filter; exposures were 7 to 10 minutes at F4·5. For further data see illustrations facing page 400. Aesculin in a gelatin medium has been used successfully to absorb ultra-violet light reflected from the object, as it allows the passage of the visible light (*i.e.*, the visible fluorescence). Certain of the glasses used for photographic work fluoresce to such an extent as to fog the plates, but H. Bäckström and R. Johansson [135] find that gelatine filters may be mounted between glass, so long as æsculin is not used.

So far back as 1910 R. W. Wood photographed the moon, using the ultra-violet light reflected from the sun, and discovered a dark patch near one of the craters; he subsequently applied the method to Jupiter and Saturn, when certain equatorial belts on the latter which had never been seen before were photographed. The method has since been applied by other workers with good results.

Ultra-Violet Light Photographs (see Frontispiece).—If the ultra-violet rays themselves are to be recorded on the plate, as is often the case in photomicrographical work, then the worker is more restricted, and the plate should be coated with the minimum amount of gelatine, and, according to Barnard,[31] should contain the maximum proportion of silver salts. Judd Lewis encountered serious difficulty in the lack of uniformity

of results from photographic X-ray plates of the same type and from the same maker. This he considers is due probably to the difference in properties between gelatine prepared in the summer and in the winter.

Plates may be sensitised to certain regions of wave-length, and thus rendered more rapid and more selective. For general work with ultra-violet light, however, ordinary plates are satisfactory. Panchromatic plates are as a rule unnecessary, since the emulsion is naturally blue-sensitive; Ilford Zenith (H and D 650) give good results, the exposure required being less for a given resolving power. For recording fluorescence, however, a panchromatic plate is required, since the colour of the luminescence is in the visible range, and ordinary plates are insensitive beyond the blue-green. Sensitised plates are used only below about 2000 Å. G. R. Harrison and P. A. Leighton [32] coated ordinary dry plates with a mixture of 1 part of a 5 per cent. gelatine solution and 2 parts of a saturated solution of æsculin, and used them for recording rays of wave-lengths between 2300 and 3700 Å. They found that this treatment tends to cut down chemical fogging, and that for work with rays of wave-length greater than 3700 Å., the æsculin may be replaced by a solution of chlorophyll. J. and J. F. Thovert [46] suggest immersing the plates in a 1 per cent. solution of citric acid or sodium salicylate, no special precautions, such as washing, etc., being required.

Messrs. N. V. Philips [50] have introduced a camera plate consisting of cellophane, pure triacetylcellulose, Sanalux glass or a glass having the percentage composition—80 SiO_2, 10 B_2O_3, 5CaO and 5Al_2O_3. Messrs. Ilford Ltd. state that plates may be sensitised to ultra-violet light by means of medical paraffin, and that such treatment extends the range to 1800 Å. The plates are bathed in a 1 per cent. solution of Nujol in pure petroleum spirit, and are then rapidly dried in a current of dry air. The layer should be so thin that its removal before development is unnecessary.

G. R. Harrison [47] has also tested the effect of coating plates with thin layers of various oils which fluoresce when illuminated with light of wave-lengths between 2200 and 3800 Å. He employed the Cramer Contrast Process, Seed 23 and Wellington Super Xtreme plates, and found that the degree of change in the sensitiveness depends on variations in the thicknesses of the oil

film and the coating. The greatest sensitiveness was found with a clear paraffin oil and a contrast plate, whilst the greatest contrast was given with a light lubricating oil.

Polished objects should be free from dust and from finger-prints. Finger-prints themselves have been photographed (*cf.* p. 228) for criminological purposes by Brose and Winson, and by Edlin (*cf.* Photograph No. 17). In these cases the finger-prints are carefully dusted with a highly-fluorescent powder, such as anthracene, which clings to the traces of organic matter left by the fingers. The excess of anthracene is removed, the prints are irradiated and photographed, and in this way photographs of finger-prints on multi-coloured backgrounds can be recorded.

The period of exposure, naturally, differs for different objects. W. Scheffer [27] photographed successfully a number of objects of academic interest in ultra-violet light and in daylight, while R. Lassé [48] has described an easy and elegant method for recording stains, etc., on textiles by means of the contact process in which short exposures only are claimed to be necessary.

The table on the opposite page gives some indication of the time of exposure, etc., required to photograph various objects. The first fourteen lines of the table refer to the mercury lamp running at 3 amperes, 85 volts, D.C., and an anastigmatic lens F 4·5 open to aperture of F 12, with Wratten and Wainwright panchromatic plates [44]; lines 15 and 16 (by C. H. Edlin) refer to a mercury lamp running at 3 amperes, Sanderson Extension Camera with lens of focal length 7 inches open to an aperture of F 7. All the figures are intended merely as a guide, as the ages of the lamp and of the filters influence to some extent the length of exposure required.

Full photographic details are also given under some of the photographs illustrated facing page 400.

The technique for development of the plates obtained is precisely the same as that employed for plates taken in the usual way, for which the reader is referred to the usual photographic journals or handbooks.

W. Scheffer [27] also found that pictures, manuscripts, cheques and other similar objects are fairly easy to photograph. Paintings often require an exposure of about 10 minutes, teeth about 30 seconds, stamps may require so long as 20 minutes, and invisible writing requires about 15 minutes, although the exact

period depends on the type of apparatus and lamp used. Good results have been obtained (by J. A. Radley and N. J. Gruber) from panchromatic plates exposed for 2 minutes behind a cerium ammonium nitrate filter or a sodium nitrite filter. Fossils generally require about 30 minutes, and the object should be very clean and free from dust or finger-prints.

TABLE 5.

No.	Object.	Lamp Filter.	Lens Filter.	Distance of Lamp from Object (Inches).	Exposure (Minutes).
1	Ceramics	Corex A	Triphenyl methane	35-49	12-20
2	Ceramics	Hanau	Triphenyl methane	33	35
3	Ceramics	Corex A	Cerium amm. nitrate	35	10
4	Glass	Corex A	Cerium amm. nitrate	35	15
5	Ivory	Corex A	Cerium amm. nitrate	35	7
6	Ivory	Corex A	Triphenyl methane	35	7
7	Ivory	Hanau	Cerium amm. nitrate	35	10
8	Marble	Corex A	Cerium amm. nitrate	30-35	7-10
9	Marble	Corex A	Triphenyl methane	35	4-8
10	Prints	Corex A	Triphenyl methane	45	10
11	Prints	Corex A	Cerium amm. nitrate	35	7-10
12	Prints	Hanau	Cerium amm. nitrate	35	10
13	Painting	Hanau	Cerium amm. nitrate	40	13
14	Textiles	Corex A	Cerium amm. nitrate	35-55	15-35
15	Forged £1 note	Wood's glass	Andrew's super protex	40	4
16	Finger-prints	Wood's glass	Andrew's super protex	40	6
17	Teeth	Hanau	Cerium amm. nitrate	40	30 secs.
18	Mildew	Wratten No. 15	—	—	10 mins.

An interesting experiment with *resonance radiation* is recorded by W. G. and P. A. Leighton.[97] Mercury vapour absorbs light of wave-lengths 2537 and 1849 Å. (resonance lines), and if resonance radiation is used to illuminate some mercury in a beaker, behind which is a screen coated with anthracene, then the shadow of the mercury vapour rising from the surface of the mercury is visible on the screen. This method should have many interesting applications. The use of rays of selected wave-lengths in the ultra-violet region has proved of great use for obtaining photographs free from background; applications of special interest occur in criminological work (see Chapter X),

particularly in connection with the photography of forged documents and finger-prints.

The next advance on this side of the subject will, it seems, be the photography of invisible fluorescence, *i.e.*, fluorescent radiations which are excited by exposure to ultra-violet light but which fall outside the visible range. The technique involved is not easy, as care must be taken to eliminate interference from other radiations, but it is gradually being perfected. Promising results of this nature have been obtained by W. R. Mansfield with ultra-violet light of relatively short wave-length as the exciting radiation.[148]

Fluorescence Microscopy.—This, which is one of the most interesting and recent applications of fluorescence as a testing method (see Grant [54]), depends on the use of ultra-violet light as a source of illumination for the ordinary microscope lamp. In this way many micro-structures are rendered visible by their characteristic fluorescence, just as in macro-work with the naked eye observation in ultra-violet light discloses features which are normally invisible. Numerous applications of the method exist and are referred to below, or in more detail in the appropriate sections in the book. The method has recently been carried a stage further by the use of fluorescent substances as differential "stains," and the "secondary fluorescence" so produced frequently enables particular details of the structure to be picked out. This is an added facility and is referred to further below.

Apparatus.—This is not elaborate, but it is of course assumed that a good microscope is available. In the first place it is useless to attempt to increase magnification unless this is accompanied by a proportionally increased resolution, and this in turn is governed by the numerical aperture of the objective and the mean wave-length of the illuminating light (with which we are principally concerned). Ultra-violet light enables both increased magnification and resolution to be obtained. The other important factor is the source of ultra-violet light, and in this connection there have been many proposals and several differences of opinion. B. K. Johnson [36] prefers a spark discharge between cadmium electrodes when quartz monochromatic microscope objectives are to be used (wave-length about 2700 Å.), whilst P. Metzner [37, 38] uses a nickel-carbon arc. Whatever source is used, however, the principal lines in the spectrum emitted should

be sufficiently separated and of maximum intensity. M. Haitinger [39] maintains (private communication) that iron electrodes produce radiations which surpass the mercury vapour lamp in intensity. The principal difficulty seems to be one of intensity. As W. Garner [55] points out, the filter used to cut out visible light usually transmits only about 20 per cent. of the ultra-violet light, and of this only a portion produces a visible fluorescence. He himself recommends cadmium- or iron-cored carbon electrodes, and successful results have been obtained by many workers with one kind or other of self-regulating carbon arc. Since a point-source is a considerable advantage, the usual form of mercury arc is far from ideal, a fact which has, however, now been appreciated by several of the manufacturers of these lamps, because, as shown in Chapter II, in certain recent designs, the radiations are concentrated so far as possible into one spot. Special reference should also be made to the magnesium electrodes advocated by J. E. Barnard and F. V. Welch.[130]

The Optical System requires a few comments, and these may conveniently be subdivided according as they refer to the use of transmitted or reflected light. In any case, however, filters and reflectors on the arc side should be made of some material which does not absorb ultra-violet light, and accordingly, ordinary optical glasses are eliminated. Quartz is best for converging lenses, condensers, etc., and it is an advantage, although not essential, to replace glass mirrors by quartz prisms or silver reflectors. According to J. J. Fox the mercury line 2537 Å. is very suitable (but little used) for this work, and it may be isolated by means of chlorine and bromine filters.

Filters to remove visible and infra-red rays which mask the fluorescence, and which heat up the specimens and lenses, respectively, should also be inserted, so that the radiation eventually obtained covers a range of about 3000 to 4000 Å.; suitable filters are described in Chapter III, a 10 per cent. solution of copper sulphate in a quartz cell about 60 mm. thick being suitable for the latter purpose. Quartz is, however, not essential, and any glass which transmits ultra-violet light is suitable and less expensive. The copper sulphate should be pure, and the solution should contain a few drops of sulphuric acid and be renewed frequently. The slide should, of course, also be made of quartz.

For work in *transmitted light* the cover-slip should preferably be opaque to ultra-violet light but transparent to visible radiation, and this serves the double purpose of protecting the eyes and of eliminating any fluorescence from the cement and the lenses in the objective, which has been known to mask the fluorescence. Goggles to protect the eye from stray ultra-violet rays are essential as a precaution against conjunctivitis if no others have been taken, and filters for insertion in the eyepiece are available for the same purpose. The usual devices for dark-ground illumination (see J. Smiles [56]) are useful adjuncts for this work, and Emich [40] has used rays which strike the object at an angle of 30° for the examination of liquids in capillary tubes. Means are sometimes

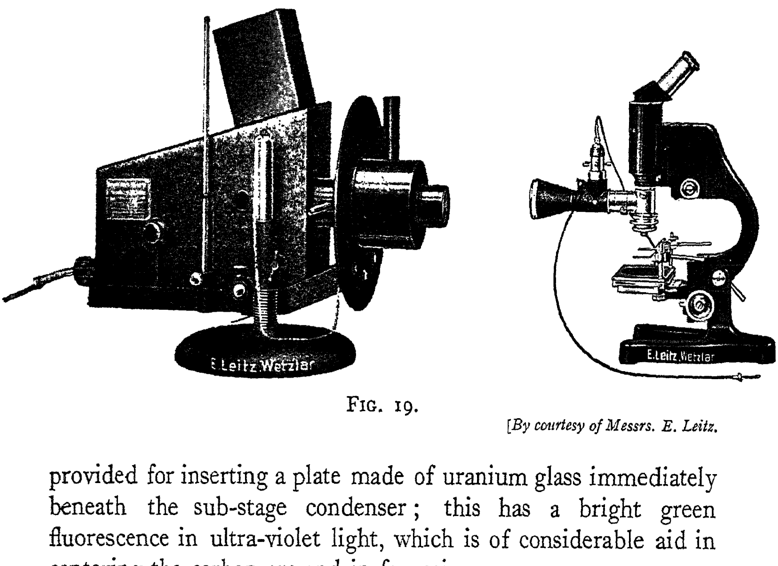


FIG. 19.

[*By courtesy of Messrs. E. Leitz.*

provided for inserting a plate made of uranium glass immediately beneath the sub-stage condenser; this has a bright green fluorescence in ultra-violet light, which is of considerable aid in centering the carbon arc and in focussing.

E. M. Chamot and E. W. Mason [41] also describe fluorescence microscopes for *dark-field illumination*. In such cases the object is self-luminous against the dark background, and every fissure, faint outline or variation in the particle which reflects light appears brilliantly contrasted. The results are very different from those obtained in transmitted light.

If *reflected light* is to be used the cover-slip should obviously be transparent both to visible and ultra-violet light, and an ordinary glass slide may be used if the ultra-violet light strikes the

object from above only. Here again there are several devices for obtaining the reflected light, a Lieberkuhn mirror with a magnification of 160°,[42] or an adaptation of the Leitz "Ultropak"[100] (see Fig. 19) being particularly efficient. A source of light of great intensity is required, and is provided by a carbon arc in the way shown, and this arrangement is also stated to minimise penetration of the ultra-violet light into the lens system. S. H. Gage[129] has found that a reflector prepared by depositing a film of aluminium vapour on a polished quartz disc is very efficient in such cases.

Photomicrography.—If other phenomena in addition to the fluorescence are to be photographed, then the lenses should preferably be made of the more expensive quartz, although with objects which fluoresce vividly photographs in colour may be taken with ordinary lenses (exposure 20 seconds). Details of procedure vary according to individual conditions (*e.g.*, nature of the specimen, source of light, etc.), but reference may conveniently be made to the section on general photographic methods (p. 69), and to the publications of M. Haitinger[131] which contain some beautiful photomicrographs of histological subjects taken by the three-colour process.

The nature of *the embedding agent*, where this is used, is of some importance, and opinions differ as to the desirability of canada balsam. Some workers maintain that its own fluorescence is sufficient to spoil the photograph, whilst others consider that samples can be obtained in which the fluorescence is weak enough to be without effect, so that the quality of the product probably is the deciding factor. Similar considerations apply to paraffin wax, although a slight fluorescence from the wax frequently throws the details of the specimen into sharper outline.

F. F. Lucas[43] has described a technique for the photomicrography of metals in which the line at 2750 Å. is used, thereby lessening the time of exposure. Other work on this branch of the subject will be found in the additional references, and recent publications by M. Haitinger[131] contain details of technique suitable for histological subjects.

Photographs Nos. 17 and 20 indicate the perfection of technique now obtainable.

General Technique.—This is similar to that used in ordinary microscopical work. A speck of quinine or anthracene (which fluoresce brightly) placed on the slide is a great aid to focussing,

and glycerol should replace cedar-wood oil for "oil"-immersion lenses; ether, carbon tetrachloride or chloroform may, however, be used for mounting purposes if the specimen is soluble in glycerol. J. J. Fox has suggested the use of menthol as a mounting-medium, since it is very transparent to ultra-violet light. Specimens (*e.g.*, in botanical work) may be stained with a mixture of barium platinocyanide and sodium salicylate and embedded in paraffin wax, and they may then even be photographed. A 5 per cent. solution of formalin is a convenient hardening-agent, and tissues, etc., may be de-pigmented by immersion in potassium hydroxide solution and exposure to ultra-violet light.[98] Finally, mention should be made of the change in fluorescence which occurs on irradiation, and which may be apparent even after 5 minutes.

Secondary-Fluorescence Microscopy.—As mentioned above, the range of structures rendered visible in ultra-violet light may often be extended considerably if the specimen is "stained" differentially with some fluorescent material, or with a non-fluorescent substance which causes the structure with which it comes into contact to fluoresce. Such substances are known by the general term "fluorochromes."

Some of the ordinary microscope stains may be used in this way, but in general it is difficult to prepare them in a sufficiently sensitive form, and their applications are limited; however, one is not necessarily restricted to such recognised stains, and fluorescent alkaloids have been found to be very satisfactory (*vide infra*). A great deal of the pioneer work in this field is due to M. Haitinger and his co-workers,[57] and they have applied it chiefly to botanical and biological work (*e.g.*, using acridine, azo dyes, diphenylamine, flavins, auramine, primulines, berberine sulphate, chelidonium- or rhubarb-extract and diamond-phosphine; see also p. 400). Stains suitable for work with fibres are described by Schulze and Göthel[58] (see p. 337), and R. Jenkins[132] gives a list of fluorochromes suitable for rendering tissues fluorescent.

The hardened specimen is sectioned in a freezing microtome and is immersed in a $1:1000$ to $1:5 \times 10^6$ solution of the stain for a period (1 to 12 hours), the actual length of which depends on the concentration and nature of the reagent used. It is then washed and mounted in non-fluorescent glycerol or liquid paraffin.

Some of the effects obtained photographically are shown in

Photographs Nos. 17, 20 and 21, and these are also reproducible in colour.

Although such methods may be used successfully for the examination of structures, care should be exercised in using the colour or intensity of the fluorescence observed as a means of diagnosis. One reason for this, viz. the change in the nature of the fluorescence on irradiation, has already been mentioned ; others are the effects of *p*H value, and the chemical or optical action on the " stain " of chemical substances or dyestuffs (*e.g.*, chlorophyll) naturally present in the preparation. An excess of formaldehyde used in the hardening operation modifies the primary fluorescence of red blood corpuscles to a blue-white colour, and it should also be remembered that many " stains " are metachromatic, *i.e.*, different colours are obtainable in daylight and in ultra-violet light (*e.g.*, thionine produces a violet and yellow colour, respectively, with nerve threads).

Applications of fluorescence microscopy are very numerous and occur in most sections of the book. Those referring to botanical work (see especially K. L. Noack,[33] S. Provazek,[34] and O. Heimstadt [35]), paper, textiles and the biological sciences will be found of considerable interest, and the Additional References on page 87 should also be consulted.

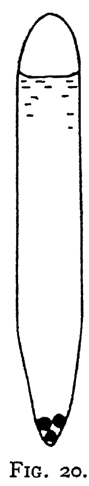


FIG. 20.

Titration Experiments.—In a number of the methods described in Part II., it is necessary to carry out titrations from a burette in ultra-violet light. As the best results are obtained in a darkened room, the problem of reading the burette is a real one. Where the liquid is sulphuric acid (*e.g.*, in the titration of quinine, p. 314) a few specks of quinine sprinkled on the surface will serve to show up the meniscus if a stray beam of ultra-violet light from the lamp strikes it. For other liquids one of us (J. Grant [45]) has used a float (Fig. 20) consisting of a piece of glass tubing, about two-thirds of the diameter of the burette, which is two-thirds filled with a solution of quinine in dilute sulphuric acid containing a little oil as froth-killer, and sealed.* The lower end may be drawn out to a point or weighted with lead-shot to keep the float vertical. The slightest illumination with

* Made by Messrs. A. Gallenkamp & Co., Ltd.

ultra-violet light causes this to fluoresce brilliantly and to illuminate, not only the meniscus, but also the graduations, so that the burette may be read in the dark accurately to within 0·05 c.c.

REFERENCES.

[1] R. W. Wood and Van der Lingen, *Proc. Royal Soc.*, 1921, **99**, 362.
[2] Niewodniczański, *Nature*, 1926, 118, 877.
[3] H. Lundén, *Zeit. angew. Chem.*, 1928, **41**, 199.
[4] F. Hein and W. Retter, *Jour. prakt. Chem.*, 1928, **119**, 368.
[5] M. Guyot, *Ann. Falsif.*, 1931, **24**, 196.
[6] M. Haitinger and V. Reich, *Chem. Fabrik.*, 1929, **379**, 529.
[7] M. Haitinger and V. Reich, *Zeit. angew. Chem.*, 1928, **41**, 982.
[8] F. M. Cale, *Trans. Roy. Soc. Canada*, 1922, **16**, 257.
[9] P. Wels, *Arch. Physiol.*, 1928, **219**, 738.
[10] J. Becker, *Münch. Med. Woch.*, 1926, **73**, 99.
[11] M. Luckiesh, " Ultra-Violet Radiation " (New York: Van Nostrand).
[12] M. Luckiesh and L. L. Holladay, *Jour. Franklin Inst.*, 1931, **212**, 787.
[13] W. Scheffer, *Photog. Industrie*, 1928, **26**, 159.
[14] Bayle and Fabre, *Bull. Soc. chim.*, 1925, **37**, 1304.
[15] A. Policard, *Bull d'Histol.*, 1926, **2**, 317.
[16] Bayle, Fabre and George, *Bull. Soc. chim.*, 1925, **37**, 89.
[17] Bayle, Fabre and George, *Chim. et Ind.*, 1927, **17**, 179.
[18] F. Perrin, *Compt. rend.*, 1924, **178**, 1978.
[19] F. Perrin, *ibid.*, 1924, **178**, 2252.
[20] L. J. Desha, *Jour. Amer. Chem. Soc.*, 1920, **42**, 1350.
[21] Morgan and MacLennan, *Biochem. Jour.*, 1928, **22**, 1514.
[22] M. Haitinger, *Mikrochemie*, 1930, 8, 81; *Pharm. Monatsh.*, 1931, **12**, 80.
[23] S. Mohorovičić, *Ark. hem. farm.*, 1930, 4, 73.
[24] H. Miethe, *Photog. Korres.*, 1927, **63**, 69.
[25] G. Kögel, *Paläontol. Zeit.*, 1928, **10**, 298.
[26] G. Kögel, " Abderhalden's Handbuch der Biologischen Arbeitsmethoden," Ab. II. (Urban and Schwarzenberg, Berlin-Wien, 1928).
[27] W. Scheffer, *Photog. Industrie*, 1928, **26**, 128.
[28] K. Weber, *Photog. Korres.*, 1930, **66**, 317.
[29] P. Metzner, *Plant. Arch. Wiss. Botan.*, 1930, **10**, 281; *Mikrochemie*, 1931, 9, 72.
[30] G. Kögel, *Mikrochem.*, 1929, **7**, 305; *Photog. Korres.*, 1930, **66**, 282.
[31] Barnard and Welch, " Practical Photomicrography " (London: Arnold & Co., 1925).
[32] G. R. Harrison and P. A. Leighton, *Phys. Rev.*, 1930, **36**, 779.
[33] K. L. Noack, *Verh. d. phys.-Med. Ges. zu Würzburg, N.F.*, 1925, **50**, 47.
[34] S. v. Provazek, *Kleinwelt*, 1914, **6**.
[35] O. Heimstädt, *Zeit. wiss. Mikros.*, 1911, **28**, 330.
[36] B. K. Johnson, *Jour. Micros. Soc.*, 1931, **51**, 268.
[37] P. Metzner, *Zeit. wiss. Mikros.*, 1928, **45**, 51.
[38] P. Metzner, " Das Mikroskop " (Franz Deuticke, Leipzig-Wien, 1928).
[39] M. Haitinger, *Mikrochemie*, 1931, 9, 220, 430, 441; 1932, **12**, 270.
[40] F. Emich, " Mikrochemisches Praktikum " (Munich: J. F. Bergmann), 1931

[41] E. M. Chamot and E. W. Mason, "Handbook of Chemical Microscopy" (Chapman & Hall), 1930.
[42] G. Kögel, *Chem. Fabrik.*, 1928, (i), 55.
[43] F. F. Lucas, *Iron Age*, 1926, **117**, 555.
[44] J. J. Rorimer, "Ultra-Violet Rays and their Use in the Examination of Works of Art" (New York, 1931).
[45] J. Grant, *Jour. Sci. Inst.*, 1932, **9**, 359.
[46] J. and J. F. Thovert, *Bull. Soc. France Phys.*, 1932, 158; through *Phot. Abstr.*, **13**, 166.
[47] G. R. Harrison, *Jour. Opt. Soc. Amer.*, 1925, **11**, 113.
[48] R. Lassé, *Melliand Textilber.*, 1930, **11**, 687.
[49] R. W. Wood, Royal Institution Lecture, 19/5/11.
[50] N. V. Philips' Gloeilampenfabr., B.P. 361,388 and 392,424, 5/10/32.
[51] H. Neugebauer, "Die Kapillar-Lumineszenz-analyse im pharmazeutischen Laboratorium" (Leipzig: W. Schwabe, 1933).
[52] R. W. Wood, Royal Institution Lecture, 19th May, 1911.
[53] J. Grant, *Nature*, 1934, **133**, 124.
[54] J. Grant, *ibid.*, **134**, 635.
[55] W. Garner, "Industrial Microscopy" (London: Pitman, 1932).
[56] J. Smiles, *Jour. Roy. Mic. Soc.*, 1933, **53**, 203.
[57] M. Haitinger and others, *Zeit. wiss. Mikros.*, 1933, **50**, 195; Abderhalden's "Handbuch der Biologischen Arbeitsmethoden," 1933, II, (3), p. 3653; *Beih. Bot. Centralbl.*, 1933, **50**, 432; *Zeit. Mikros. Anatom. Forsch.*, 1933, **33**, 193.
[58] B. Schulze and E. Göthel, *Pap. Fabrik.*, 1934, **32**, 110; *Zellstoff u. Pap.*, 1934, **14**, 93.
[59] M. Guyot, *Ann. Falsif.*, 1931, **24**, 196.
[60] M. Haitinger, *Mikrochemie*, 1932, **11**, 429.
[61] H. Kautsky, A. Hirsch and W. Baumeister, *Ber.*, 1931, **64**, 2053, 2677; 1932, **65**, 401.
[62] A. Tschirch and E. Stock, "Die Harze" (Berlin: Borntraeger, 1933).
[63] J. Eisenbrand and G. Siewert, *Arch. Pharm.*, 1934, **272**, 428, 440.
[64] S. J. Wawilow and T. A. Tummermann, *Zeit. Phys.*, 1929, **54**, 270.
[65] K. Weber and M. L. Savić, *Zeit. Phys. Chem.*, 1934, **24B**, 68.
[66] W. Buchloh, *Zeit. Russ. Physiol.*, 1933, **6**, 166.
[67] P. W. Danckwortt, *Chem.-Ztg.*, 1933, **57**, 1018.
[68] P. Ernst, *Zeit. Math. Natur. Unters.*, 1930, **68**, No. 8.
[69] R. Intonti, *Giorn. Chim. Ind. Appl.*, 1932, **14**, 23.
[70] J. Mansell, *Inst. World*, 1928, **1**, 178.
[71] J. H. Frydlander, *Rév. Prod. Chim.*, 1932, **35**, 321.
[72] R. E. Liesegang, *Zeit. Wiss. Mikros.*, 1926, **43**, 517.
[73] H. Schneider, *Pharm. Zentr.*, 1933, **74**, 379.
[74] E. Haschek and M. Haitinger, *Mikrochemie*, 1933, **13**, 55; *Chim. Ind.*, 1933, **30**, 1401; *Sitzungsber. Akad. Wiss. Wien.*, 1931, **141**, 621.
[75] W. L. Lewschin, *Zeit. Phys.*, 1931, **72**, 382.
[76] H. Kautsky, A. Hirsch and W. F. Davidshöfer, *Ber.*, 1932, **65**, 1762.
[77] G. Klein and H. Linser, *Biochem. Zeit.*, 1930, **219**, 51.
[78] H. R. Hirst, *Jour. Soc. Dyers and Col.*, 1931, **47**, 347; 1934, **50**, 211.
[79] J. C. Lennan, *Proc. Roy. Soc. London*, 1923, **102A**, 256 (Æsculin).
[80] F. M. Cale, *Trans. Roy. Soc., Canada*, 1922, **16**, 257 (Æsculin).
[81] R. W. Wood, *Phil. Mag.*, 1922, **43**, 757 (Dyes).
[82] C. Moureu, C. Dufraisse, and P. M. Dean, *Compt. rend.*, 1927, **184**, 285.
[83] D. L. Gamble and G. F. Stutz, *Jour. Ind. Eng. Chem.*, 1929, **21**, 330 (Synthetic resins).

[84] F. Wischo, *Pharm. Monatsh.*, 1930, **11**, 224.
[85] J. Becker, *Munch. Med. Wöch.*, 1926, **73**, 99.
[86] D. N. Chakravarti and N. R. Dhar, *Zeit. Anorg. Allg. Chem.*, 1925, **142**, 299 (Æsculin).
[87] K. G. Mathur and S. S. Bhatagnar, *Ind. Jour. Phys.*, 1928, **3**, 37.
[88] E. H. Kranck, *Soc. Sci. Fennica Comment. Phys. Math.*, 1929, **5**, No. 1.
[89] A. Petrikaln, *Zeit. Physik.*, 1927, **42**, 435.
[90] F. Perrin, *Compt. rend.*, 1927, **184**, 1097 ; 1931, **192**, 1727 ; " La Fluorescence des Solutions " (Diss., Paris, 1929).
[91] C. Dhéré, Abderhalden's " Handbuch der Biologischen Arbeitsmethoden," II, 3, 4 (Berlin : Urban and Schwarzenberg), 1933 ; " La Fluorescence en Biochimie " (Paris : Presses Universitaires), 1937.
[92] A. A. Dixon, *Jour. Opt. Soc. Amer.*, 1931, **21**, 250.
[93] S. J. Wawilow, *Zeit. Phys.*, 1925, **31**, 750.
[94] J. Grant and H. Procter-Smith, *Analyst*, 1934, **59**, 749.
[95] J. Bouchard, *Compt. rend.*, 1934, **199**, 460.
[96] L. Bendikson, *Library Jour.*, 1932, **57**, 789 ; 1933, **58**, 243 ; 15 Sept., 1934 ; 1 Jan., 1936.
[97] W. G. and P. A. Leighton, *Journ. Chem. Ed.*, 1935, **12**, 139.
[98] G. Hollister, *Zoologica*, 1934, **12**, No. 10 ; through *Nature*, 1934, **134**, 779.
[99] O. Vodrážka, *Zeit. wiss. Mikros.*, 1929, **46**, 497.
[100] A. H. Willoughby, *Proc. Tech. Sec. Papermakers' Assoc.*, 1935, **16**, 114.
[101] W. Grassmann and O. Lang, *Collegium*, 1935, 114, 401.
[102] F. Bandow, *Zeit. Phys. Chem.*, 1938, **39B**, 155.
[103] E. R. Bolton and K. A. Williams, *Analyst*, 1938, 63, 84.
[104] H. Thaler, *Zeit. Unters. Lebensm.*, 1938, **75**, 130.
[105] G. Meister, U.S.P. 2,013,417, 2,013,584, 3/9/35.
[106] L. H. Lampitt, N. D. Sylvester, and P. Bilham, *Biochem. J.*, 1935, **29**, 1167.
[107] F. J. Kaiser, *Rec. Trav. chim. Pays-Bas*, 1938, **57**, 117.
[108] A. Thiel, *Biochem. Zeit.*, 1937, **294**, 221.
[109] H. Funk and H. Steps, *Zeit. tech. Phys.*, 1934, **15**, 301.
[110] H. Steps, *Fortschr. Röntgenstr.*, 1935, **52**, 293 ; *Chem. Zentr.*, 1936, (i), 301.
[111] F. H. Cohen, *Rec. Trav. chim. Pays-Bas*, 1935, **54**, 133.
[112] E. J. Bowen and J. V. Sawtell, *Trans. Faraday Soc.*, 1937, **33**, 1425.
[113] F. Duschinsky, *Angew. Chem.*, 1936, 49, 647.
[114] O. Maercks, *Zeit. Phys.*, 1938, **109**, 685.
[115] W. West, R. H. Müller and E. Jette, *Proc. Roy. Soc.*, 1928, **121A**, 294, 299, 313.
[116] R. Toussaint, *Compt. rend.*, 1931, **193**, 933.
[117] A. Thiel and W. Thiel, *Chem. Fabrik.*, 1932, **5**, 409.
[118] K. Weber, *Zeit. phys. Chem.*, 1931, **15B**, 18 ; *ibid.*, 1932, **19B**, 22, 30.
[119] J. Wawilow, *Zeit. Physik*, 1925, **31**, 750 ; **32**, 239.
[120] H. Linser, " Fluorometrie. Handbuch der Pflanzanalyse " 1, Kap. 11.
[121] H. Linser, *Biochem. Zeit.*, 1932, **244**, 157.
[122] G. Klein and H. Linser, *ibid.*, 1930, **219**, 51.
[123] G. Klein and H. Linser, *Arch. wiss. Botanik*, 1932, **15**, 767.
[124] P. Wels and M. Jokisch, *Pflüger's Arch.*, 1928, **219**, 738.
[125] P. Wels and M. Jokisch, *ibid.*, 1931, **228**, 672.
[126] H. Hellström, *Arkiv. Kemi. Min. Geol.*, 1937, **12A**, No. 17, 12.
[127] H. Minibeck, *Biochem. Zeit.*, 1937, **293**, 219.

[128] F. Bandow, *ibid.*, 1938, **295**, 154.
[129] S. H. Gage, "The Microscope," 16th Edn., 1936.
[130] J. E. Barnard and F. V. Welch, *J. Roy. Microscop. Soc.*, 1936, **56**, 361, 365; 1937, **57**, 256; "Photomicrography," 3rd Edn., 1936.
[131] M. Haitinger, Abderhalden's "Handbuch der Biologischen Arbeitsmethoden," 1934, II., (3), p. 3307; *Chem.-Ztg.*, 1937, **61**, 847; "Fluorescenz Mikroskopie" (Leipzig: Akademische Verlagsgesellschaft), 1938.
[132] R. Jenkins, *Stain Techn.*, 1937, **12**, 167.
[133] G. C. Brock, *Sci. Proc. Roy. Dublin Soc.*, 1933, June.
[134] A. Boutaric and J. Bouchard, *Jour. Phys. Radium*, 1937, **8**, 1.
[135] H. Bäckström and R. Johansson, *Zeit. Wiss. Phot.*, 1937, **36**, 194.
[136] H. W. Meyer, *Leica Photography*, 1937, April.
[137] L. Bendikson, *Library J.*, 1936, Jan. 1st; *Analyst*, 1936, **61**, 213.
[138] Anon., *Hanovia Tech. Brochure*, S. 2300.
[139] W. M. Thornton and M. N. Lewis, *Bull. Soc. Franc. Minér.*, 1934, **57**, 268.
[140] J. Bouchard, *Jour. Chim. Phys.*, 1936, **33**, 232.
[141] J. Bouchard, *ibid.*, 1936, **33**, 325.
[142] H. Hellström, *Naturwiss.*, 1935, **24**, 76.
[143] K. Weber, *ibid.*, 1936, **24**, 318.
[144] A. Boutaric and J. Bouchard, *Ann. Soc. Sci. Bruxelles*, 1936, **56B**, 35; *Jour. Chim. Phys.*, 1936, **33**, 325; (with C. Achard), *Compt. rend.*, 1936, **201**, 629.
[145] K. Weber, *Zeit. Physikal. Chem.*, 1935, **30B**, 69.
[146] J. Weiss, *Nature*, 1935, **136**, 794; 1936, **138**, 80; (with H. Fischgold), *Zeit. Physikal. Chem.*, 1936, **32B**, 135.
[147] L. A. Tumerman, *Compt. rend. Acad. Sci. U.R.S.S.*, 1935, **1**, 471.
[148] W. R. Mansfield, *Medico-Legal and Crim. Rev.*, 1939, **7**, 29.
[149] *British Journal of Photography*, Almanac (London: H. Greenwood).
[150] A. Schischlovski, *Zavod. Lab.*, 1938, **7**, 760.

ADDITIONAL REFERENCES.

R. Lassé, *Melliands Textilber.*, 1931, **12**, 249 (Object carrier for fluorescence analysis of textiles).

G. S. Haslam and C. H. Hall, *Jour. Opt. Soc. Amer.*, 1934, **24**, 14 (Microscopical study of particle-size of paint-pigments).

M. Haitinger and H. Hamperl, *Zeit. Mikros. Anatom. Forsch.*, 1933, **33**, 193 (Fluorescence microscopy of animal tissues).

P. Ellinger and A. Hirt, *Zeit. Anat. u. Entwicklungsgesch.*, 1929, **90**, 791; *Arch. Exp. Path. Pharm.*, 1929, **145**, 193; 1930, **150**, 285 (Fluorescence microscopy of living organisms).

P. Ellinger and W. Koschara, *Nature*, 1934, **133**, 553 (Fluorescence microscopy of animal pigments).

F. Querner, *Zeit. Mik. Anat. Forsch.*, 1933, **32**, 444 (Medicine and Histology).

E. Wagner, *Paläontol. Zeit.*, 1928, **10**, 298 (Museum work).

J. Plotnikow, *Brit. Jour. Photog.*, 1931, Dec. 25; *Photog. Korres.*, 1931, **67**, 256 (Photography in infra-red and ultra-violet).

E. Jurgens, *Photog. Korres.*, 1929, **65**, 220 (General photographic work in ultra-violet).

F. F. Lucas, *Science*, 1930, **71**, 515; *New Intl. Assoc. Test. Mat.*, Sept., 1931 (Optical sectioning in U.V. microscopy).

O. Mente, *Zeit. f. Reproduktionstecknik*, 1913, **15**, 42, 58 (Photography).

H. C. Jaquet, *Photog. Korres.*, 1928, **64**, 203 (Photography).

Köhler, *Zeit. wiss. Mikros.*, 1904, **21**, 163 (Microphotography).

H. Stübel, *Arch. Physiol.*, 1913, **149**, 1.

H. Schrötter, *Wien Med. Woch.*, 1922, **72**, 1199.

König, *Vh. phys.-med. Ges.*, *Würzburg*, 1927, **49**, 160.

W. Walkhoff, *Ges. Morph. u. Physiol.*, *München*, 1922, **33**, 7 ; 1924, **49**, 159 (Physiological photography in ultra-violet light).

B. T. Squires and J. H. Jeffree, *Jour. Sci. Inst.*, 1928, **5**, 273 (Fluorophotometer).

L. C. Martin, *ibid.*, 1928, **5**, 380 (Ultra-violet microscopy).

A. Köhler, *Naturwiss.*, 1933, **21**, 165 (Photomicrography).

T. H. Daugherty and E. V. Hjort, *Jour. Ind. Eng. Chem. Anal.*, 1934, **6**, 370 (Apparatus for fluorescence microscopy).

O. Eichler, *Cellulosechem.*, 1934, **15**, 114 ; 1935, **16**, 1 (Study of lignins by fluorescence microscopy).

M. Haitinger, *Mikrochemie*, 1935, **16**, 321 (Use of the fluorescence microscope in microchemistry).

J. Grant, *Chem. and Ind.*, 1938, **43**, 496 (General technique and recent advances).

W. R. Mansfield, *Phot. J.*, 1935, April, 169 (Photography).

E. Haschek and M. Haitinger, " Farbmessungen," 1936 (Measurement of fluorescence).

Eastman Kodak Co., " Photographic Plates for use in Spectroscopy and Astronomy," 1935 (Photography).

Eastman Kodak Co., " Wratten Light Filters," 1936.

J. T. Randall, *J. Roy. Soc. Arts*, 1937, **85**, 353 (General).

J. Grant, *Chem. and Ind.*, 1937, **42**, 237 (General).

J. Grant, *Photography*, 1937, **6**, 12 (Photography).

J. Plotnikow, *Chem. Ztg.*, 1931, **55**, 318 (Photography).

J. A. M. van Liempt and P. Leydens, *Rec. Trav. Chim. Pays-Bas*, 1937, **56**, 26 (Response of various photographic plates to ultra-violet light).

A. Kufferath, *Silk and Rayon*, 1937, **11**, 520 (Photomicrographic apparatus).

H. Langenbruck, *Arch. für Kriminologie*, 1938, Nos. **1** and **2**, 102 (Ultra-violet reflex photomicrography).

Anon, *Quartz Lamp*, 1938, 47 (General).

H. Freytag, *Photog. Korres.*, 1935, **71**, No. 4 (Development of half-tones on textiles using ultra-violet light).

H., F. G. A., *Quartz Lamp*, 1938, 47 (General).

B. Luyet, *Compt. rend.*, 1935, **200**, 1997 (Study of cytoplasm and caryoplasm by ultra-violet photomicroscopy).

R. Herman-Montague and L. Herman, *Sci. ind. phot.*, 1936, **7**, 225 (Photographic plates sensitised with sodium salicylate).

F. F. Lucas, *Ind. Eng. Chem.*, 1938, **30**, 146 (Ultra-violet microscopy).

B. K. Johnson, *J. Sci. Inst.*, 1934, Dec. (Ultra-violet microscope for opaque objects).

C. J. Frosch and E. A. Hauser, *Ind. Eng. Chem. Anal.*, 1936, 8, 423 ; *J. Opt. Soc. Amer.*, 1937, **27**, 110 (Fluorescence microscopy in industrial research, with special reference to the study of emulsions).

P. A. Mattis, *Amer. J. Pharm.*, 1936, **103**, 276 (Fluorescence microscopy applied to the endocrine glands).

F. Himmelweit, *Lancet*, 1937, Aug. 21 (Fluorescence microscopy of living virus with oblique incident illumination).

M. Servigne, *Ann. Chim. Applic.*, 1937, **17**, 313 (Review of uses and bibliography of the analytical applications of fluorescence microscopy).

[20] R. Nesini, *Prag. Arch. f. Tiedmed.*, 1933, **12**, 237.

[21] R. Nesini, *Tierärzt. Rundschau*, 1933, **39**, 266.

[22] Anon., *Imp. Bur. Plant Genetics (Aberystwyth), Bull.*, No. 11, 1934.

[23] Anon., *Nature*, 1933, **131**, 446.

[24] F. Mach and P. Lederle, *Phosphorsäure*, 1932, **2**, 623.

[25] R. Nesini, *Zeit. Unters. Lebensm.*, 1934, **67**, 195.

[26] J. Voss, *Angew. Bot.*, 1934, **16**, 510; 1936, **16**, No. 6.

[27] P. W. Danckwortt, *Zeit. Unters. Lebensm.*, 1935, **69**, 458.

[28] J. Grant, *Fert. Feeding Stuffs and Farm Supplies*, 1935, **22**, 674.

[29] Pieper and Grumbach, *Deut. Landw. Presse.*, 1932, **59**, 183; Geyer, *Die Kartoffel*, 1932, **12**, 109; T. Marx and E. Merkenschlager, *Landw. Jahrbuch.*, 1932, **76**, 733.

[30] T. P. McIntosh, *Gardener's Chron.*, 1936, **6**, 13; 20/6/36.

[31] A. Salmony, *Dairy Ind.*, 1938, **3**, 129.

[32] E. Bottini, *Ann. Sperim. Agrar.*, 1934, **15**, 61.

[33] R. Illgen, *Deut. Landwirtschaft*, 22/4/33.

PART II.

APPLICATIONS OF FLUORESCENCE ANALYSIS.

CHAPTER I.

AGRICULTURE.

THE uses of the lamp for the control of foods are discussed on page 141, and have a certain amount of bearing on its applications in the farm [31, 32] (see also Botany, p. 104, and Bacteriology, p. 97). C. von Wahl [1] has published a paper dealing with the control of *feeding-stuffs*, such as bee-food, cow-cake, etc.; also with seeds, animal droppings and other familiar substances of farm life. Samples of *manure*, artificial and otherwise, can be compared with the bulk of the manure delivered, and if so desired, with artificial manures or *fertilisers*, and it is found that whilst particles of bone meal fluoresce with a bright blue colour, di- and tri-calcium phosphates and superphosphates appear only a dull violet. Most phosphates, in fact, fluoresce to some extent, and it is therefore possible to distinguish the basic slag from that from the Thomas process (" Thomas meal ") which does not.[24] Other work on phosphatic fertilisers is referred to under slags (p. 111) and in Chapter VIII. If anthracene is mixed with a fertiliser it is possible to determine when the latter is evenly distributed by examining the fertilised soil in ultra-violet light.[23]

Seeds.—F. Albrecht [2] has used the lamp with some success to differentiate between various strains of *barley* which appear to be the same in ordinary light. The grains appear green, blue or violet, and can therefore be divided into groups differing in chemical and biological characteristics (*cf.* p. 168). A. Niethammer,[3] who has investigated the fluorescence of fresh sound seeds and of old or damaged seeds, finds that the use of the lamp allows one to differentiate to some extent between these two classes, but

that the results serve as a surer guide when applied to the identification of different types. His results are tabulated below :—

Pea (*Pisum sativum*).	Lilac fluorescence, with red streaks which are absent from old samples.
Scarlet runner (*Phaseolus vulgaris*).	Lilac fluorescence, absent from old samples.
Bean (*Vicia sativa*).	Bright green fluorescence, absent from old samples.
Linseed (*Vicia faba*), and Bean (*Linum usitatissimum*).	Dark blue or lilac to pale yellow according to the age of the sample.
Walnuts. Hazels. Almonds.	Lilac fluorescence when fresh, changing to yellow on ageing.
Hemp (*Cannabis sativa and Ervum lens*).	Green fluorescence. Old samples appear matte white.

R. Nesini [20, 21, 25] states that the normal fluorescence of *peas* is replaced by black or bright yellow spots if bacteria or moulds produced by storage in a damp place are present ; a sorting method based on the selective effect of this radiation on a photo-electric cell has been devised by R. Horsfield, but no details are available (see also Photograph No. 23, facing p. 400).

Niethammer obtained no differentiation between new and old samples of corn-cockle, black or white mustard, rye, wheat, barley or fagopyram since they all give shades of purple or lilac. J. Tausz and H. Rumm,[4] however, have found that *wheat* falls into four groups showing different colour reactions under the lamp (see Voss,[26] and p. 168). Members of the first group fluoresce with an intense blue colour, those of group two have a weak blue fluorescence, group three an intense green, whilst wheats in the fourth group are devoid of fluorescence except for a dull brownish colour ; 25 per cent. of the grains investigated were found to fall in Group III. There appears to be no difference in water-content between members of Groups I and III, but on storage, the green fluorescence of the latter gradually changes to the blue fluorescence characteristic of the former. Investigations on the grains of barley, maize and oats indicated that wheat is alone in this behaviour. L. Karácsonyi [17] has examined the fluorescence of corn and has extracted the fluorescing substance with various solvents. The addition of acids to these extracts produced no change in the colour of the fluorescence, but alkalis developed characteristic colours ranging from blue to green. E. Berliner and R. Rüter [18] (see also Haitinger and Reich [14]) have also used the lamp for differentiating between infected and sound seeds,

especially of wheat and rye. P. Barbade [19] has confirmed that vetch has a yellow to reddish fluorescence whilst that of ergot is yellow-orange, but P. W. Danckwortt [27] failed to find any correlation between the fluorescence and the toxicity of the cyanogenetic glucosides from the former seeds. Seed spices are discussed under Food (p. 168).

Poultry Foods.—J. Grant and H. Procter-Smith have examined a large number of such products. The most striking effects were obtained with fine cut wheat, dari, barley groats, broad-bran and maize meal, which gave a vivid white fluorescence, often with a tinge of blue. Ground oats, on the other hand, appeared dull brownish-white, and Dutch blue peas showed as dull purple with vivid bluish-white or yellow patches.

Whole seeds (*e.g.*, hemp, sunflower seeds and pea nuts) usually gave an indefinite brown colour, but when cut the centres showed up with a vivid fluorescence in various shades of blue and white. Limestone grit, charcoal and oyster shell grit gave negative results, and it was possible to estimate roughly the amounts present in mixtures with fluorescent grains. Although tares in the whole seed gave no fluorescence, the crushed seeds were vivid golden-yellow, and 2 per cent. were easily distinguishable in mixtures with wheat (see Photograph No. 1, facing p. 400). Maples behaved similarly, being pale-yellow under the skin, although the centres were white with yellow or bluish tinges. The results of such examination enable anyone familiar with the method to identify and estimate the ingredients of such mixtures.

Filter-Paper Analysis.—L. Linsbauer [12] has also examined a great number of seeds, particularly in cross-section, under the lamp. Leguminous seeds, in particular, present many coloured fluorescence effects, so that in addition to a differentiation between various species of each genus, a distinction may often be found between different strains of the same species. This worker employed the method of placing the seedlings to be examined on discs of filter-paper moistened with tap-water, and allowed them to germinate in a tank such as is ordinarily used in routine seed testing. The seeds are put down in four, six or eight batches of 50 seeds per filter-paper disc, and after 4 to 6 days, when the roots have begun to develop, they are examined under the lamp, the number of seedlings conveying

fluorescence to the filter-paper being expressed as a percentage of the total number present.

It appears to be essential to use white, non-fluorescent and ashless filter-paper in this method, since the fluorescence is not obtainable on porous blocks, or on sand or glass. Other papers that have not undergone too extensive a commercial treatment will also answer the purpose, but black filter-papers give no results, since the black pigment masks the fluorescence or protects the paper from the root exudate. The chemistry of the fluorescence has not as yet been examined, but it may be suggested that the cellulose of the filter-paper is partly hydrolysed by the exudate from the roots with the production of a fluorescent compound. If the various products of hydrolysis of cellulose are examined in order of their formation, it is found that soluble or insoluble starch (commercial type) is not fluorescent, the dextrin produced at the next stage being fluorescent, whilst dextrose in the following stage is also non-fluorescent. It seems, therefore, that the root exudate carries the hydrolysis to a stage at which traces of dextrin are produced. On the other hand, the appearance of the fluorescence may be merely a phenomenon connected with the formation of an adsorbate in a colloidal system somewhat on the same lines as in the case of the fluorescence of chlorophyll mentioned on page 299. These suggestions are mere speculations.

If the roots are split a faint fluorescence is observed in those which give the fluorescent exudate, but it is not nearly so brilliant as that which appears on the filter-paper. The roots of cereals fluoresce brightly even when grown in sand, but this is not the case with rye-grass (see Photograph No. 12, facing p. 400).

Rye-Grass.—A number of papers have been published by S. P. Mercer and P. A. Lineham [6-9] dealing with the application of the method to the study of rye-grass seedlings, and their results are in close accordance with those of N. R. Foy,[10] who has also worked on the same problem. The distinction of the seeds of perennial rye-grass (*Lolium perenne*, L.) from those of Italian rye-grass (*Lolium multiflorum*, Lam.) is not at all difficult, providing the terminal awns of the Italian species are present, but it is practically impossible when the seeds have been machine-dressed, owing to the removal of the awns in this process (see Photograph No. 12, facing p. 400). By means of the first

method mentioned above these workers found that the seedlings of Italian rye-grass and some of those of false perennials give a blue fluorescence which is absent in the case of the true, normal perennial rye-grass. Not many of the seeds develop a fluorescence after 12 days, but Mercer and Lineham obtained the reaction in some cases after 18 days if they removed the non-fluorescent seedlings to a fresh filter-paper after each examination. Their final conclusion is that the degree of fluorescence is a measure of agricultural value.[16]

Fluorescence occurs in all species of *Lolium*, and appears, usually, to be associated with a short life-factor and, according to L. Corkill,[15] to be inherited according to a single Mendelian character; it is faint blue in colour but sometimes disappears after a few days. F. Nilsson[11] obtained similar results with commercial samples of Italian rye-grass, but found that in certain cases, where the plants were artificially self-fertilised, a number of non-fluorescent seedlings occurred among the progeny. Nevertheless, he also concludes that Italian rye-grass seedlings are normally fluorescent. He also reports a general absence of fluorescence in *Lolium perenne*, although he found a number of exceptions among named strains, and observed both fluorescent and non-fluorescent seedlings among the progeny of some self-fertilised plants.

Fluorescence may be observed in Western Wolths (*L. multiflorum* Lam.), darnel (*L. remotum* Schrank, and *L. Brazilianum* Mes.), while Foy has also noted it in Wimmera rye-grass from New South Wales. The method therefore provides a useful laboratory procedure to distinguish seed samples of the valuable Hawkes Bay strain from the inferior strains grown in other districts of New Zealand, and is recommended by the Imperial Bureau of Plant Genetics at Aberystwyth.[22]

Oats give an intense violet-blue fluorescence on the filter-paper, and the rootlets of the *Danthonia* species, *Bromus hordeaceus* L., and *Bromus uniloides* H.B.K., show a similar though less intense fluorescence. It will be seen that for several reasons the method may not yet be regarded as strictly quantitative for the determination of the percentage of superior strains present, but it certainly provides a reliable qualitative test or means of diagnosis of plant type.

G. Gentner's work[5] on *Phaseolus vulgaris* has shown that

a number of bacteria and fungi which grow on seeds during storage often show bright-coloured fluorescence effects, although these colours generally disappear when the seeds are dried.

Reference to work on *potatoes* is made below,[29, 33] but special attention should be drawn to the observation of T. P. McIntosh [30] who found that the variety Golden Wonder had an electric blue fluorescence, whilst the fluorescence of other varieties was red-purple in colour.

According to L. V. Dodds [13] *tobacco* is examined in industry for bacterial contamination and other damage, and when cigarettes are examined under the lamp the impurities often show, even through the paper, by the gleaming white fluorescence which they emit.

Nux vomica is sometimes added to cattle-cake as a purgative, and such additions have to be regulated carefully on account of the alkaloids present. The drug can readily be distinguished under the lamp and control is thereby greatly simplified. Further work on agricultural products has been published by V. Reich and M. Haitinger,[14] and by J. Grant.[28]

REFERENCES.

[1] C. von Wahl, *Fortschr. d. Landswirtschaft*, 1928, 3, 979.

[2] F. Albrecht (see Foods, p. 141).

[3] A. Niethammer, *Zeit. Unters. Lebensm.*, 1929, 57, 354.

[4] J. Tausz and H. Rumm, *Zeit. ges. Mühlengewesen*, 1928, 5, 115.

[5] G. Gentner, *Prakt. Blatter f. Pflanzenbau u. Pflanzschutz*, 1929, 6, 166.

[6] S. P. Mercer and P. A. Lineham, *Bull. Ministry Agric. Gov. N. Ireland*, July 25, 1930.

[7] P. A. Lineham and S. P. Mercer, *Bull.*, No. 17, Ministry Health and Fisheries, London.

[8] P. A. Lineham and S. P. Mercer, *Proc. Roy. Dublin Soc.*, 1931, 20, 75.

[9] P. A. Lineham and S. P. Mercer, *Proof Report*, Sixth International Seed Testing Congress, Wagenigen, Holland, 1931.

[10] N. R. Foy, *New Zealand Jour. Agric.*, 1931, 43, 389.

[11] F. Nilsson, *Botaniska Notiser*, Lund, 1930.

[12] L. Linsbauer, *Botan. Archiv*, 1929, 23, 441.

[13] L. V. Dodds, *Contemporary Rev.*, 1930, No. 773, 638.

[14] V. Reich and M. Haitinger, *Fortschr. d. Landswirtschaft*, 1928, 3, 433; *Mühle*, 1929, 66, No. 41.

[15] L. Corkill, *Nature*, 1932, 130, 134.

[16] P. A. Lineham and S. P. Mercer, *ibid.*, 1933, 131, 203, 474.

[17] L. Karácsonyi, *Zeit. ges. Getreide- u. Mühlenwesen*, 1932, 19, 69.

[18] E. Berliner and R. Rüter, *ibid.*, 1929, 5, 203.

[19] P. Barbade, *Ann. Falsif.*, 1931, 24, 581.

[20] R. Nesini, *Prag. Arch. f. Tiedmed.*, 1933, **12**, 237.
[21] R. Nesini, *Tierärzt. Rundschau*, 1933, **39**, 266.
[22] Anon., *Imp. Bur. Plant Genetics (Aberystwyth), Bull.*, No. 11, 1934.
[23] Anon., *Nature*, 1933, **131**, 446.
[24] F. Mach and P. Lederle, *Phosphorsäure*, 1932, **2**, 623.
[25] R. Nesini, *Zeit. Unters. Lebensm.*, 1934, **67**, 195.
[26] J. Voss, *Angew. Bot.*, 1934, **16**, 510 ; 1936, **16**, No. 6.
[27] P. W. Danckwortt, *Zeit. Unters. Lebensm.*, 1935, **69**, 458.
[28] J. Grant, *Fert. Feeding Stuffs and Farm Supplies*, 1935, **22**, 674.
[29] Pieper and Grumbach, *Deut. Landw. Presse.*, 1932, **59**, 183 ; Geyer, *Die Kartoffel*, 1932, **12**, 109 ; T. Marx and E. Merkenschlager, *Landw. Jahrbuch.*, 1932, **76**, 733.
[30] T. P. McIntosh, *Gardener's Chron.*, 1936, **6**, 13 ; 20/6/36.
[31] A. Salmony, *Dairy Ind.*, 1938, **3**, 129.
[32] E. Bottini, *Ann. Sperim. Agrar.*, 1934, **15**, 61.
[33] R. Illgen, *Deut. Landwirtschaft*, 22/4/33.

CHAPTER II.

BACTERIOLOGY.

Bacteria.—Probably the first workers on the fluorescence of bacteria were Arloing, Policard and Langeron,[1] who, however, did not study the subject deeply, but only devised a rough differentiation between various bacteria. R. Gassul and A. Zolkovic [2] stated later that every species of bacterium has a characteristic fluorescence in filtered ultra-violet light, but their conclusions have been modified by E. Danielson,[3] who found that whilst a number of distinct species give characteristic fluorescence colours, in some cases different strains of the same species show different colours. In many cases the intensity of the fluorescence is a function of the oxygen pressure.[18]

The *p*H value of the nutrient media may affect the fluorescence; thus P. Lasseur and co-workers [21] find that *Bacillus caryocyaneus* (types S and Rb) in an asparagine medium having an initial *p*H value of 4·3 to 6·5 show a fluorescence maximum after 3 days, followed by a decrease. If, however, the intial *p*H value of the medium is below 6·2, the fluorescence tends to become more blue or violet, but at higher *p*H values the colour becomes green with the Rb type. Type S tends to give a green fluorescence, as it hydrolyses more of the asparagine and so produces a medium of higher final *p*H value than is the case with the Rb type. The variations in colour are possibly due to the formation of varying amounts of two pigments having a blue-violet and yellow-green fluorescence, respectively. The effect of the *p*H value of various reagents on the fluorescence of the colouring matters produced during normal metabolism by *B. pyocyaneus*, *B. fluorescens* and *B. putidus* has been studied by F. Giral [24] who has separated by chromatographic analysis two pigments responsible for the fluorescence. A green fluorescent pigment has also been separated from *Pyocyaneus fluorescens* by C. E. Turfitt.[25]

The procedure followed for the examination of bacteria under the lamp is to plate-out the cultures in the usual way, and after incubation, to transfer the colonies on a platinum wire to a piece of damp, white filter-paper in a Petri dish. The specimen is then dried in the oven at a low temperature and examined under the lamp. As most Petri dishes and culture media themselves fluoresce, this technique is necessary in order to obtain the fluorescence of the colonies free from interference from other fluorescent bodies. Cultures for comparison should be of the same age and should have been grown on the same medium, and the Petri dishes may conveniently be covered with a sheet of cellophane or similar substance transparent to ultra-violet light.

R. J. V. Pulvertaft [15] states that cultures should preferably be aerobic and grown on a non-fluorescent meat medium containing only the initial breakdown-products of pancreatic extracts. The fluorescent substances are best dissolved in absolute alcohol or in acetone, as they are only sparingly soluble in ether or chloroform. They are non-volatile and are stable to boiling water or to hot 0·05 *N* hydrochloric acid, but not to warm 0·05 *N* sodium hydroxide.

Results obtained with various types of the *paratyphoid bacterium* and the Flexner and Shiga types of *dysenteris bacterium* are shown in Table 6A (for meat-peptone on an agar basis), whilst in Table 6B, the fluorescence colours of the acid groups, which

TABLE 6A.

Species.	*Fluorescence Colour.*
Bact. paratyphi-A.	Bright yellow with violet tinge.
Bact. paratyphi-B.	Greenish-yellow.
Bact. paratyphi-N_1.	Cream colour with greenish lustre.
Bact. paratyphi-N_2.	Bright yellow.
Bact. Dysenteriae Flexner.	Pale cream, somewhat yellowish.
Bact. typhi abd.	Pale cream, like *B. Dys. Flexner.*
Bact. coli.	Yellow-white.
Bact. Dysenteriae Shiga	White, with a bright blue lustre.
Bact. faecale arom.	Luminous yellow, with a greenish tinge.

TABLE 6B.

Species.	*Fluorescence Colour.*
Bact. tuberc. hum.	Yellowish-rose.
Bact. Leprae (Kedrowsky).	Pale rose.
Bact. tuberc. avium.	Delicate bright rose.
Bact. smegmae.	Luminous rose.
Bact. Rabinowitsch.	Rose-cream.

appear yellow in daylight but show various rose-coloured shades of fluorescence under the lamp, are given for a medium consisting of potato in 6 per cent. glycerin.

Although it is difficult to describe subjective observations under the lamp, such differences can be seen quite plainly after very little practice. This is especially the case with milks inoculated with cultures of *Streptococcus Cremoris* and *Streptococcus Lacticus*. In the first case the milk shows a bluish-white fluorescence under the lamp, whilst in the second, the fluorescence is more intense, and is white with a bluish tinge, the controls (*i.e.*, uninoculated milk) having a less intense ivory-white colour (Radley and Gruber).

Definite differences in fluorescence colour were found even with pigment-producing bacteria such as *B. pyocyaneus* and *Sarcina lutea*. *B. Tobler-I*, for example, has a deep rose-red fluorescence, *B. Grassberger-II*, matte dark red, and *B. leprae* (*Duval*) an ochre colour. In the case of *B. Chlororaphus*, with which is associated the pigment chlororaphine (phenazine-α-carboxamide), C. Dhéré [12] showed that when the pigment is converted into dihydrophenazine-α-carboximide by reduction with sodium hydrosulphite or with zinc dust and acetic acid, the normal faint green fluorescence changes to brilliant orange. The chlororaphine formed from this by re-oxidation of an aqueous solution in air is, however, non-fluorescent. In another instance described by L. B. Pett,[19] the change in fluorescence colour of a lactoflavin (from green to blue) was found to be due to a non-sporing, gram-negative bacterium which was absent from other flavin-containing compounds such as brewers' yeast.

H. O. Hettche and W. Vogel [26] find that *B. pyocyaneus* shows a strong bactericidal action against *B. fluorescens*. With young, but not with old cultures, this action is proportional to the amount of pigment produced. J. G. Eymers and K. L. von Schouwenburg [28] find that the quantum efficiency of the light-emitting process in *Photobacterium phosphoreum* is a function of the temperature, being a maximum at 22° C., while I. M. Korr [27] finds that heat treatment does not lower the intensity of the fluorescence unless the organism is damaged. L. B. Pett [29] considers from fluorescence evidence that moulds and many species of bacteria do not appear to contain lactoflavin.

Finally, the work of F. Labrousse [13] on phytopathogenic

bacteria may be mentioned, because it indicates the importance of the composition of the medium. He found that *B. Tabacum* and *B. Medicagnis* (var. *phaseolicola*) grown on a beef-extract medium show a definite fluorescence, but only if magnesium and a phosphate are present (see above).

C. Dhéré [14] has (with his co-workers) evolved a suitable technique for the application of spectrographic methods to bacteriological and similar problems (*cf.* p. 248).

A group of Japanese workers [5, 6] has carried out some interesting work on the luminescence from the fresh-water shrimp, *Xiphocardine compressa, de Haan*, and have isolated highly luminous bacteria to which they have given the name of *Microspira phosphoreum*. Merker (p. 104) refers to marine organisms which impart a fluorescence to water containing them, and this is attributed to renal secretions (see Plotnikow [16]).

According to S. E. Hill [7] the penetration of various liquids into bacteria can be followed from the progressive extinction of their luminescence. The cells are not injured either by $H^{\cdot}$ or OH' ions in the surrounding solution within the range of *p*H values provided by the presence of ammonium salts, and Hill therefore concluded that in isotonic solutions of such salts the disappearance of the luminescence must be due to the penetration of the solute.

Fish and meat products when examined under the lamp often show a marked fluorescence, or a change in fluorescence, due to bacterial action, and the method therefore appears to be promising for the investigation of the keeping properties of fish and meat that has to be transported over a distance. Additions of inferior portions (*e.g.*, sinews) also may often be detected. Colours due to moulds on *peas* are discussed on page 91 (Agriculture), and mildew on textiles is illustrated in Photographs Nos. 16 and 22 (facing p. 400).

It might be expected that *sera* varying in nature and origin would show corresponding variations in fluorescence colours. According to S. S. Okuniewski and L. Penska,[9] however, this is not the case, since the fluorescence of all sera examined appeared uniformly blue in colour, but acquired a green or brown tinge when infected with organisms; traces of cholesterol produced no change. These observations, if confirmed, might provide a useful foundation for the examination of sera.

A consideration of the *bactericidal action of ultra-violet light* lies outside the scope of this work, but it should be mentioned that ultra-violet light of short wave-length may alter the stain-reactions of certain organisms,[20] and that some fluorescing compounds seem to have the power of assisting the lethal action of the rays. H. Welch and R. G. Perkins [10] found that very long exposures to the rays do not kill all the bacteria present, but the addition of fluorescein and eosin in dilutions of 1 in 10,000 to 1 in 1,000,000, renders *B. coli* organisms so sensitive to the rays that 100,000 per c.c. are completely destroyed after 4 seconds of exposure. With concentrations of 1 in 10,000,000 and 1 in 100,000,000 the action was the same as in the absence of the dyes, whilst with concentrations of 1 in 100 and 1 in 1000 the dyes absorb the rays and so render them ineffective. The process is considered of value for the disinfection of orange and grape fruit juices, and so is probably capable of being extended to other foodstuffs. L. V. Beck and A. C. Nichols [22] find that fluorescent basic dyestuffs are more toxic and have a greater photosensitising action towards *Paramecia* at *p*H 7·4 than at *p*H 6·2. Fluorescent acid dyestuffs, however, are more effective at *p*H 6·2. According to G. Valette [23] the fixation of quinine on *Paramecium caudatum* is a maximum in the neighbourhood of the digestive vacuoles.

G. Rossetti [11] has confirmed the results of Techoueyres, namely, that doses of less than 0·3 mgrm. of free chlorine per litre exert a vigorous disinfecting action, and they both consider that the action is due to the emission of ultra-violet light.

Fungi.—Certain mould fungi show violet shades of fluorescence which, so far, have not been observed with bacteria. The examination of the fluorescing pigment of certain *Actinomycetes* has been carried out by F. Cortese,[4, 8, 17] who studied, in particular, the influence on the fluorescence of anaerobic culture, and of the *p*H value and chemical constitution of the culture medium. He thence concluded from the fluorescence of *A. albus* and *A. sulphureus Gaspherini* that they are different species of *A. bovis* Harz, the pink-red fluorescence given by meat cultures of *A. albus* being characteristic of this species. The fluorescent material was extracted from the medium with weak alcohol and purified by shaking with acidified ether, and was then found to be an amorphous, odourless, reddish-brown substance belonging

to the group of porphyrins (see p. 249), and showing a marked fluorescence in a dilution of 1 in 640,000.

A. E. Oxford [30] finds that " mycoporphyrin " obtained from the mould *Penicilliopsis Clavariaeformis* contains no nitrogen and should therefore be termed " penicilliopsin." It has no fluorescence in neutral solvents, but can be oxidised to oxypenicilliopsin which is fluorescent in neutral solution but only after exposure to daylight for a short time. The spectrum of this fluorescence has been examined by C. Dhéré and V. Castelli.[31] Emodin itself also gives a purple oxidation product under the same conditions, and Dhéré and Castelli [32] find that its fluorescence spectrum contains the characteristic red bands of oxypenicilliopsin.

REFERENCES.

[1] Arloing, Policard and Langeron, *Compt. rend. Soc. Biol.*, 1925, **101**, 261.
[2] R. Gassul and A. Zolkovic, *Zentr. Bakt. Parasitenkunde*, 1927, **104**, 503.
[3] E. Danielson, *Zentr. Bakt.*, 1929, **115**, 225.
[4] F. Cortese, *Boll. soc. Ital. Biol. Sper.*, 1930, **5**, 423, 842.
[5] Y. Yoshiwo, *Sei-Ikwa Zasshi* (Jap.), 1926, 45.
[6] Yasaki Yoshiwo, Masao Nishio, Akira Ichikawa, Ritsuji Majima and O. Ishikawa, *ibid.*, 1926, 45.
[7] S. E. Hill, *Jour. Gen. Physiol.*, 1929, **12**, 863.
[8] F. Cortese, *Boll. ist sieriter*, Milan, 1931, **10**, 77.
[9] S. S. Okuniewski and L. Penska, *Compt. rend. Soc. Biol.*, 1931, **107**, 289.
[10] H. Welch and R. G. Perkins, *Jour. Preventive Med.*, 1931, **5**, 173 ; *Zbl. ges. Hyg.*, 1931, **26**, 6.
[11] G. Rossetti, *Boll. soc. Ital. Biol. Sper.*, 1931, **6**, 102 ; *Chim. et Ind.*, 1932, **26**, 1338 ; Abstr. No. 589, by *Water Pollution Research Board*, 1932, **5**, 151.
[12] C. Dhéré, *Compt. rend. Soc. Biol.*, 1934, **115**, 1461.
[13] F. Labrousse, *Compt. rend.*, 1932, **194**, 2173.
[14] C. Dhéré, S. Glückmann and L. Rapetti, *Compt. rend. Soc. Biol.*, 1933, **114**, 1250 ; C. Dhéré, Abderhalden's " Handbuch der Biologischen Arbeitsmethoden," Abt. II, Teil 3, Heft 4, p. 3097 ; M. Haitinger, *ibid.*, p. 3307 (Berlin : Urban and Schwarzenberg), 1933.
[15] R. J. V. Pulvertaft, *Jour. Path. Bact.*, 1934, **38**, 355.
[16] J. Plotnikow, *Biol. Zentr.*, 1931, **51**, 441 (Marine fauna).
[17] P. Redaelli and F. Cortese, *Raggiultraviol.*, 1930, **6**, 52 (*Actinomycetes* and *Eumycetes*).
[18] H. Shapiro, *Jour. Cell. Comp. Physiol.*, 1934, **4**, 313.
[19] L. B. Pett, *Nature*, 1935, **135**, 36.
[20] A. J. Allen, R. F. Franklin and E. McDonald, *Phys. Rev.*, 1933, **43**, 1041.
[21] P. Lasseur, A. Dupaix-Lasseur and W. Palgen, *Trav. lab. microbiol. faculté. pharm. Nancy*, 1937, **10**, 23.
[22] L. V. Beck and A. C. Nichols, *J. Cell. comp. Physiol.*, 1937, **10**, 123.
[23] G. Valette, *Compt. rend.*, 1935, **201**, Oct. 14th.
[24] G. Giral, *Anal. Fís. Quím.*, 1936, **34**, 667.
[25] G. E. Turfitt, *Biochem. J.*, 1937, **31**, 212.

[26] H. O. Hettche and W. Vogel, *Arch. Hyg. Bact.*, 1937, **117**, 234.
[27] I. M. Korr, *J. Cell. comp. Physiol.*, 1935, **6**, 181.
[28] J. G. Eymers and K. L. van Schouwenburg, *Enzymologia*, 1937, **1**, 328.
[29] L. B. Pett, *Biochem. J.*, 1935, **29**, 937.
[30] A. E. Oxford, *J. Soc. Chem. Ind.*, 1938, **57**, 975.
[31] C. Dhéré and V. Castelli, *Compt. rend. Soc. Biol.*, 1937, **126**, 1061.
[32] C. Dhéré and V. Castelli, *ibid.*, 1938, **128**, 815.

ADDITIONAL REFERENCES.

O. Schaumann, *Arch. exp. Path. Pharm.*, 1930, **151**, 197 (Rôle of rivanol in the organism).

P. Lasseur, A. D-Lasseur and R. Lécaille, *Trav. Lab. Microbiol. Fac. Pharm., Nancy*, 1931, **4**, 85 (Bacterial cultures).

F. R. Georgia and C. F. Poe, *Jour. Bact.*, 1931, **22**, 349; 1932, **23**, 135 (Influence of inorganic constituents of culture media on fluorescence of bacteria).

F. Gewehr, " Untersuchungen über den Resistenzerhöhenden Einfluss von Bakterien ' Schutzstoffen ' in Desinfektionsversuchen " (Diss., Gottingen, 1930).

S. Kitamura, *Jap. Jour. Dermatol.*, 1932, **32**, 253 (Pathogenic moulds).

F. M. Clara, *Mem. Cornell Univ. Agric. Expt. Stn.*, No. 159, 1934 (Pathogenic plant bacteria).

H. Hellström and H. von Euler, *Mikrochem., Molisch Festschr.*, 1936, 209 (Chromosomes examined in ultra-violet microscope).

A. C. Giese and P. A. Leighton, *Science*, 1937, **85**, 429 (Fluorescence of cells and cell products).

C. Dhéré, *Schweiz. Med. Woch.*, 1938, **68**, 948 (Fluorescence methods in microbiology).

G. M. Lewis, *Arch. Derm. and Syph.*, 1935, March (Fluorescence of fungi).

M. Doudoroff, *Enzymologia*, 1938, **5**, 239 (Bacterial luminescence and lactoflavin).

A. von M. Haupt, *Strahlenther.*, 1938, **61**, 636 (Production of fluorescent substances by micro-organisms).

CHAPTER III.

BOTANY.

In filtered ultra-violet light, as indicated in the previous section, many *plant substances* show a more or less characteristic fluorescence, in some cases even at high dilutions, although this may disappear when the *p*H value falls outside a certain range. G. Klein and H. Linser [1] have studied the reaction of a number of plant substances and plant tissues under the lamp, both macroscopically and microscopically. They first fixed the fluorescent substances, in many cases by treatment with sodium hydroxide solution, when an intense green fluorescence often appeared from *e.g.*, cell-walls, wood-starches and a number of sugars. A number of three-carbon compounds, such as pyruvic acid, show the green fluorescence in alkaline solution without further treatment. The tissues were found to have a fluorescence similar to that of sugar, and the behaviour of both tissues and sugar with change of *p*H value was found to be similar (a change in fluorescence being noted at *p*H 7·0). On the appearance of the green colour the fluorescence-intensity was found to increase by many times its original amount. E. Merker [24] has found that plants impart a fluorescence to water in which they are immersed (*cf.* pp. 100 and 378).

H. H. Strain [27] has isolated two fluorescent substances from the leaves of many plants, and from etiolated maize and barley seedlings, by extraction with light petroleum and then subjecting the extract to chromatographic analysis on magnesium oxide. He considers that these two substances may play a part in the vital processes of the plant. By lixiviating tobacco with concentrated solutions of alkali and extracting the solutions obtained with ether, A. P. Forjaz [28] considers it is possible to characterise the tobacco used.

The fluorescence of the organs of *Aesculus hippocastinum* during its growth period was also studied by these workers,

and a fluorescent glycoside was found, chiefly in the inner bark-sheath and bast-fibres, without exception in the bud scales and in the pith crown, and occasionally in the pith. It seems that the glycoside remains in the organ unassimilated, and when the seed sprouts the fluorescent material always makes its appearance around the embryo ; young organs show a green fluorescence, the origin of which is not quite clear.

H. Lehmann and Professor Ambronn [2] have also investigated the fluorescence of a number of *botanical specimens* under the lamp. In microtome sections cut through the stalks and immersed in water, the peripheral cells, which are filled with wax, glow with a bright yellow colour. The cells on the outer surface fluoresce with a blue colour, and the chlorophyll glows with its characteristic red colour (see p. 299), whilst the centre of the section has a few or none of these red fluorescent cells. Various woods were examined and the cambium rings were found to be distinctly recognisable, although woods cannot be differentiated one from another in this way (*infra*). Thin sections through a cucumber stalk or the stalk of a pond lily give striking results and are worthy of special note. The latter shows the cellulose network as an intense, bright blue fluorescence, while enclosed in the network are portions of the cell-membrane, fluorescing with a brown colour, and also the fluorescent red grains of chlorophyll. R. Nuccorini and M. Monsacchi [13, 14] have shown by comparative experiments that the juice of *Chelidonium majus* and common berberis have a similar yellow fluorescence, whilst the juice of olive leaves and solutions of olenitole have a similar blue fluorescence. These workers have examined further the fluorescence of substances from the olive tree, black cabbage, rape, rue, sweet clover, lettuce and spinach, and conclude that the substances responsible for it are probably pigments which are formed as secondary products of the biochemical activity of the plants.

F. Bruno [15] has examined the green pigment of funiculus and of the immature seeds of *Yacaranda mimosae folia*, and concludes that it is not due to chlorophyll, as it has a different absorption spectrum ; also, the fluorescence of the funiculus pigment is violet, that of the seed pigment is pale red, whilst that of chlorophyll is fiery red. The capillary method has been used by a number of workers for the examination of crude plant juices and extracts [16-19] (see also p. 58) ; and further work on plant pigments

has been carried out by K. G. Stern,[20, 21] who has isolated hepto-flavin from horse liver by Kuhn's method (see Foods). F. H. Cohen [29] has extracted a substance by adsorption on franconite which has a strong blue fluorescence and which is not a decomposition product of lactoflavin, and G. Brooks [30] has determined the fluorescence spectra of ashes obtained from the tissues of *Rana esculenta* in alcohol and in concentrated sulphuric acid solution.

For the examination of *fruits*, a thin section is cut and placed between a microscope slide and cover slip. Thus, with tomato the flesh cells glow with a weak blue colour and are interspersed with a yellowish fluorescing plasmic substance, whilst the skin appears blue. Another beautiful demonstration object is the juice of the elderberry which contains the red grains of chlorophyll and other cells which glow with a bright blue fluorescence. Photographs Nos. 20 and 23 (facing p. 400) illustrate the effects obtainable with certain fruits. E. Bottini [31] has determined the substances responsible for the fluorescence of essences or extracts of citrus fruit and finds that methyl-anthranilate is responsible for the blue fluorescence from oranges, citropentene for that of lemons, and bergapentene for that of bergamots. The essences from the cortical glands contain yellow fluorescent pigments, but Bottini considers that if the cortical glands are avoided only blue fluorescent compounds would be extracted (see also H. Nicol [37]). Bruises on fruit may be detected, according to G. Haussmann,[32] by their fluorescence, the oils expressed by surface damage having a yellow fluorescence, while deep-seated injuries are revealed by the violet fluorescence of the juice.

Woods.—O. Vodrážka [3] and C. D. Mell [25] have examined and photographed the fluorescence of some woods. The polished surface of the cross-section of the stem of *Robinia* shows up the yellowish-green fluorescence of the heart wood whilst the sap wood appears blue. The annual rings are also distinctly visible since the spring-wood fluoresces differently from summer-wood. The large vessels and medullary rays can also usually be distinguished by their fluorescence, since fluorescent substances are formed within the vessel walls and reach the rest of the wood via the medullary rays. They can be extracted with alcohol or with acetone, and the solution so obtained fluoresces with the same colour as the wood itself. A blue fluorescence is given by the whole of *Ailanthus*

wood, and with *Rhus* the cork fluoresces crimson, the bark light blue, the sap wood blue, the heart wood yellowish-green, and the pith brick-red. O. Eichler [38] has used the lamp to distinguish between various kinds of wood, and to examine the distribution of liquors and incrustants in them.

Further work on the fluorescence of woods has been carried out by H. Wislicenus,[4] who makes special mention of the yellow fluorescence shown by acacia wood. When woods are frozen, only the inner rind of the twigs appears brown in filtered ultra-violet light, whilst with the normal living twig the whole rind shows a characteristic fluorescence. The rind of dried twigs shows but little fluorescence in this region, and it is therefore assumed by M. Haitinger and his co-workers [5] that freezing produces greater desiccation than evaporation. A. Künemund [8] used the lamp to examine the original lignified layers of *Salix alba*, and found that the original wood shows a moderately intense fluorescence which gradually increases ; Wasicky [9] has also mentioned that even slight lignification can be detected by this method. A. Nowak [10] has shown that various portions of the wood can be differentiated, and that diseased wood shows a white fluorescence which allows differentiation from sound wood. O. Eichler [26] used fluorescence microscopy to study the woody membranes of mono- and di-cotyledons and of gymnosperms, particularly from the point of view of the stages of lignification. He was thus able to demonstrate the formation of intermediate products (amyloids and collose) in monocotyledons, and to study the " skeleton " of pure lignin obtained after hydrolysis with hydrofluoric acid. Further work on woods has been carried out by R. Lassé [12] (see also additional references). The fluorescence of woods is dealt with from the industrial aspect in Chapter XVI on Paper, etc.

The various fluorescence colours of *grasses and seeds*, and those obtained on filter-paper when the latter are allowed to germinate on it are discussed under Agriculture (see p. 90). P. Metzner [6] makes the further observation that nearly all seeds and fruits liberate a fluorescent substance when they are placed in water and allowed to swell.

M. Haitinger and L. Linsbauer [11] give tables showing the effects of the best of fifty dyes used for staining sections, and correlate them with the time of staining, concentration, etc., for sections of

epidermis, hypodermis, parenchyma, resin ducts, vascular bundles, etc., of *Pinus niger* and *Allium* (see Photograph No. 23, facing p. 400). Mauri [7] has shown that edible varieties of mushrooms fluoresce yellow or grey, and poisonous varieties brown, while further work on this branch has been carried out by G. B. Mafei.[22] The mushrooms *Lactarius deliciosus* have been examined in detail by H. Willstaedt [33] who extracted repeatedly with alcohol, ether being added to the extract followed by sufficient water to cause the formation of two layers. By chromatographic adsorption two substances were separated, one of which had a violet fluorescence. The aqueous portion of the extract contained lactoflavin. Several workers [35, 36] have examined the fluorescence of algæ, and O. Roche [23] has investigated the fluorescence of the pigments of the red and blue algæ, phycoerythrin and phycocyanine, respectively (see p. 379). C. Dhéré and V. Castelli [34] have separated the orange pigment pencilliopsin from the mycelium of *Penicilliopsis clavariaeformis* ; they consider that it is a polyhydroxyanthraquinone which, on exposure to light, becomes fluorescent (*cf.* p. 128).

R. J. Best [39] has examined the effect of certain *plant diseases* on the appearance of the plants in ultra-violet light. The primary lesions of tomato spotted wilt (T.W.S.) virus in tobacco leaves show bright fluorescent haloes extending into the apparently-unaffected green mesophyll. As the lesion develops so the halo spreads, and it appears to be due to a water-soluble and fairly stable organic compound. It exists in small quantities in healthy plants, but it is produced in much larger amounts when the plant is infected. The leaves of tomato plants infected with tobacco mosaic virus do not contain demonstrable amounts of the substance, and it is not developed as a result of infection of all host plants. Further, the reaction is not specific for T.W.S. virus, but appears to have a wider significance which is connected with injury to the tissues.

A similar effect is noted around the primary lesions of tobacco mosaic and T.W.S. viruses on *N. glutinosa*, of T.W.S. virus in petunia and potato leaves, but rarely round secondary T.W.S. lesions on tomato leaves. Nasturtium leaves infected with T.W.S. do not show it at all.

The fluorescence has been used to trace the path of the virus in tobacco leaves after inoculation with T.W.S. virus.

The fact that the path of a fluorescent substance in a plant can be readily followed under the lamp has been used by Rhodes [40] to study the movement taking place in plants, fluorescein being the tracing agent. The use of fluorescent compounds as stains in microscopy (see p. 82) is of particular significance in botanical work, and H. Döring [41] finds that living cells show a fluorescence differing from that of dead cells when both are stained with eosin, and this indicates a difference in the physico-chemical union between cell and dyestuff. Fluorescein is taken up by the nucleus, plasma and plastid starch from acid solution and by the vacuoles from alkaline solution.

REFERENCES.

1 G. Klein and H. Linser, *Osterreich Bot. Zeit.*, 1930, **79** (2), 125.

2 H. Lehmann, *Phys. Zeit.*, 1912, **13**, 35; *Zeit. wiss. Mikrosk.*, 1913, **30**, 417.

3 O. Vodrážka, *Zeit. wiss. Mikrosk.*, 1930, **46**, 497.

4 H. Wislicenus, *Zeit. angew. Chem.*, 1928, **41**, 1315.

5 M. Haitinger, L. Linsbauer and A. Eibl, *Biochem. Zeit.*, 1929, **215**, 191.

6 P. Metzner, *Biochem. Zeit.*, 1930, **224**, 448.

7 G. Mauri, *L'Ind. Ital. d. Alimentari*, March, 1932.

8 A. Künemund, *Bot. Arch.*, 1932, **34**, 462.

9 R. Wasicky, "Fluoreszenzerscheinungen," Handbuch der prakt. u. wiss. Pharmazie (Berlin-Vienna: Urban & Schwarzenberg, 1924).

10 A. Nowak, *Zeit. angew. Chem.*, 1930, **43**, 93.

11 M. Haitinger and L. Linsbauer, *Bot. Centralblatt*, 1933, **50**, 432.

12 R. Lassé, *Mikrokosmos*, 1931, **24**, 187.

13 R. Nuccorini and M. Monsacchi, *Boll. ist. super. agrar. Pisa*, 1930, **6**, 252.

14 R. Nuccorini, *ibid.*, 1932, **8**, 481.

15 F. Bruno, *Lavori ist. botan. Palermo*, 1933, **4**, 155.

16 H. Valentin, *Apoth. Ztg.*, 1933, **48**, 1008.

17 C. A. Rojahn and H. Herzog, *Pharm. Ztg.*, 1929, **74**, 987.

18 C. A. Rojahn and H. Filsz, *ibid.*, 1932, **77**, 829.

19 W. Peyer, *Apoth. Ztg.*, 1930, **45**, 670.

20 K. G. Stern, *Ber.*, 1933, **66**, 555.

21 K. G. Stern, *Nature*, 1933, **132**, 784; 1934, **133**, 178.

22 G. B. Mafei, *Atti dell Inst. bot. dell 'universita di Pavia*, 1932, February, Vol. 3.

23 O. Roche, *Arch. Phys. Biol. Chim. Phys. Corps organisés*, 1933, **10**, 91.

24 E. Merker, *Naturwiss.*, 1931, **19**, 433; *Zoöl. Jahrb. Physiol.*, 1931, **49**, 69.

25 C. D. Mell, *Textile Col.*, 1927, **49**, 29.

26 O. Eichler, *Cellulosechem.*, 1934, **15**; 114; 1935, **16**, 1.

27 H. H. Strain, *Nature*, 1936, **137**, 946.

28 A. P. Forjaz, *Rév. Chim. pur. appl.*, 1933, **8**, 8.

29 F. H. Cohen, *Chem. Weekblad*, 1935, **32**, 441.

30 G. Brooks, *Compt. rend.*, 1937, **205**, 1465.

31 E. Bottini, *Ann. Sper. agrar.* (*Rome*), 1934, **15**, 61.

[32] G. Haussmann, *ibid.*, 1934, **15**, 55.
[33] H. Willstaedt, *Ber.*, 1935, **68B**, 330.
[34] C. Dhéré and V. Castelli, *Compt. rend. Soc. Biol.*, 1937, **126**, 1061.
[35] D. Vermeulen, E. C. Wassink and G. H. Reman, *Enzymologica*, 1937, **4**, 258.
[36] C. Dhéré and A. Raffy, *Compt. rend. Soc. Biol.*, 1935, **119**, 232.
[37] H. Nicol, *Perfum. Essent. Oil Rec.*, 1935, **26**, 85.
[38] O. Eichler, *Cellulosechem.*, 1934, **15**, 114; *ibid.*, 1935, **16**, 1.
[39] R. J. Best, *Austral. J. exp. Biol. med. Sci.*, 1936, **14**, 199.
[40] Rhodes, *Proc. Leeds Phil. Soc.*, 1937, **3**, 389.
[41] H. Döring, *Ber. deut. bot. Ges.*, 1935, **53** 415.

ADDITIONAL REFERENCES.

O. Klemm, "Fluoreszenz-Erscheinungen im Pflanzenreich," Diss. Jena, 1917.

O. Vodrážka, *Sbornik Czechoslav. Acad. Zemedelske*, 1930, **5**, 1.

G. Klein and H. Linser, *Zeit. wiss. Biol., Abt. E. Planta Arch. wiss. Botan.*, 1932, **15**, 767 (Glycosides).

G. Klein and H. Linser, *Biochem. Zeit.*, 1930, **219**, 51 (Estimation of æsculin).

A. Wrzesinska, *Polish Acad. Sci.*, 4/2/35 (Plant trypaflavins).

A. Kautsky and R. Eberlein, *Naturwiss.*, 1938, **26**, 576 (Changes in the fluorescence of marine algæ in sea water on excitation with ultra-violet light).

P. Lobert, *Bull. Soc. Chim. Biol.*, 1938, **20**, 766 (Chromatographic technique for investigating barley rootlets.

See also Drugs, p. 117, and Agriculture, p. 90.

CHAPTER IV.

CONSTRUCTIONAL MATERIALS AND GLASS, ETC.

Slags.—A number of investigators have already examined the possibilities of ultra-violet rays for testing the products of the *silicate industry*, and so far the results have been fairly encouraging. Guttmann,[1] for example, has investigated the appearance of iron blast-furnace *slags* under the lamp, and finds that stable slags, that is to say, those which do not disintegrate on storage, show a dark violet fluorescence on their freshly-broken surfaces. After weathering yellowish-white spots appear on the violet background. Unstable slags, on the other hand, show numerous clusters and spots of a red to yellow or a cinnamon-brown colour on the violet background. These brownish spots are attributed to particles of the γ-dicalcium silicate which are formed from the α- and β-forms with a corresponding increase of 10 per cent. in volume.

Basic slags, according to F. Mach and P. Lederle [7] (see p. 90), do not fluoresce, but owing to the brown or vivid yellow fluorescence of raw phosphates (especially from Florida or Algeria) 10 per cent. of these are easily detectable in the slags. Extracts in water give unreliable results, but if 10 per cent. hydrochloric acid is substituted, the characteristic fluorescence is usually obtained. Extracts in ether or in alcohol are also strongly fluorescent, but this is due to the presence of organic matter, *e.g.*, lubricating oil derived from grinding processes.

Road Engineering Materials.[17]—H. B. Milner [6] has obtained only poor results with the identification of asphalts as such, although he was able to derive valuable technical information from examinations of solutions in various solvents, or of stains on paper. Aggregates, fillers and tars were also studied.* In

* Bitumen, pitch, etc., are further dealt with under FUELS (Chapter VII), and the Chapters on MINERALS AND GEMS and on MUSEUM WORK should also be consulted.

a private communication he states: "The methods (given on p. 190) have not yet been extended to the examination of the mixtures used in road-making. From the analytical point of view, unknown mixtures are likely to present considerable difficulty in identification. The fluorescence, for instance, of Trinidad Lake asphalt would be profoundly modified by the addition of flux oil, the exact effect depending on the source of the flux oil. Without a knowledge of all the ingredients, it is doubtful whether even a qualitative analysis of the mixture could be carried out by ultra-violet ray analysis.

"Attempts have been made by other workers to apply ultra-violet analysis to the quantitative examination of unknown mixtures, such as the determination of tar in tar-bitumen mixtures. Our experience is that the method may lead to serious inaccuracies for the reasons stated above. Tar-bitumen mixtures containing the same percentage of tar may have widely different fluorescences according to the type of tar incorporated, since, although tars have a much stronger fluorescence than asphaltic bitumens, the colour and intensity varies considerably. Horizontal retort-, vertical retort- and oil gas-tars are usually easily distinguished, although borderline cases occur where the distinctions are not so obvious. Filtration of the free carbon increases the intensity of the fluorescence, but does not impair, to any great extent, the distinctions between the different types of tar."

The subject has also been investigated by D. M. Wilson [16] and by R. Grün and G. Kunze [2] who worked on road and track slags. Slag cements have been studied by V. Kubelka [3] who states that a number of these do not fluoresce, but that Portland cement shows a light brown fluorescence. The fluorescence of pigmented cements is best studied in linseed oil pastes, but it should be remembered that it is liable to be affected by the presence of calcium or barium sulphate.

The *weathering of rocks* has been examined in ultra-violet light by A. Schmölzer [4] who tested specimens of stone taken from a church in Vienna (see also Chapter XIII). Small splits, cleavages and mica particles were easily visible under the lamp. A method of investigating the *degree of penetration* of binding materials (such as bitumen in road making), and of stone-hardening preparations, is to saturate the rock with some fluorescent material such as a mineral oil, and then to examine a broken face of the

stone under the lamp. The penetrating material shows up brightly against the dull background of the stone, and the amount of penetration may easily be gauged.

The corrosion products of aluminium are dealt with by Carlsohn and Voigt,[14] but apart from this, it cannot be said that fluorescence analysis has found many applications in the study of metals. There appears, however, to be further scope in this direction, because, as the work of J. Smiles and H. Wrighton [19] has shown, the use of ultra-violet radiation in photo-micrography enables a more detailed knowledge to be obtained of the fine structure of metals and alloys, *e.g.*, sorbitic and martensitic structures in nickel-chromium steels. When a quartz objective (N.A., 1·25) suitable for a wave-length of 2650 Å. was compared with a visible light objective (N.A., 1·60), much sharper results were obtained. In this way F. F. Lucas [27] was able to reveal minute quenching cracks of the order of 25 and 1000 atom-diams. in width and length, respectively.

The possibilities of ultra-violet rays and X-rays in the *ceramic industry* have been examined by O. Krause,[5, 11] who mentions that it should be possible to follow the influence of oven-gas on the glaze and body of an article, and to differentiate between clays and kaolins. Work in the U.S.S.R. (communicated by N. Tschernjavsky,[22] C. Philipp [23]) has shown that Portland puzzuolana and other *cement* mixes may be graded in terms of strength by means of their fluorescence, comparisons being made against a standard colour-scale with the aid of a photo-electric cell. The method is in routine use in an institute for testing building materials at Dnepropetrovsk, U.S.S.R.[23]

Glass.—Apart from museum work (p. 278) and filters (p. 25), few investigations have been published on fluorescence analysis as applied to glass. It is known definitely, however, that the method is in use in the laboratories of certain optical glass specialists ; in addition, the work of B. E. Cohn [8] on borate glasses containing zinc and manganese indicates a possible application, since he found that the intensity of the fluorescence was a maximum when the ratio Zn/Mn was 23 : 1. W. B. Lester [9] has found it possible to differentiate between glasses coloured with manganese and selenium, and has also indicated further applications of this nature, notably in the cases of glasses containing manganese with cobalt or calcium. According to M. Guillot,[10] iridescence in glass is

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due to calcium carbonate formed by the presence of sodium bicarbonate, but it is also usual to add oxides or selenides of alkaline earth metals, zirconium, uranium, etc., to produce luminescence.[13, 15] F. Eckert and K. Schmidt [18] have been able to trace a connection between the yellow-brown fluorescence of glasses containing cerium and the cerium content. It is, however, also affected by the presence of other substances (*e.g.*, arsenic stimulates it, but boric acid inhibits it), and fusion in an oxidising medium also increases it. The time of exposure to the radiation is also a factor determining the nature of the fluorescence. Such glasses also show a thermoluminescence. P. Gilard, L. Dubrul, F. Jamar and D. Crespin [25] have endeavoured to find which elements are responsible for the fluorescence of glasses. Few generalisations can, however, be drawn from the mass of experimental data they record, because some elements (*e.g.*, aluminium, calcium or zinc) produce fluorescence in certain non-fluorescent glasses (*e.g.*, potassium silicate glasses) but not in others (*e.g.* sodium silicate glasses). Other elements (*e.g.*, lead) produce fluorescence in both cases, while the colour of the fluorescence produced by sulphur, boron or selenium depends on the nature of the other elements present.

However, there appears to be considerable scope for further investigations on applications of the method to glasses. Thus, for example, optical glasses of the same type (*e.g.*, lenses) but from different manufacturers show unmistakable differences in fluorescence ranging over a series of colours from pale yellow to violet, blue and vivid white; in particular, "protective" glasses of the Crookes type are characterised by a bright blue-white colour. The differences in shade are very subtle, but they allow of little doubt, and since it is very unusual for glasses from different sources to have the same appearance, the method is in use by one firm for identifying its own and other products. The source of these differences is as yet unknown, but minute traces of rare earths have been suggested as a possible explanation, and it certainly is known from the work of O. Deutschbein [21, 26] that the presence of certain rare earths in some glasses in small quantities produces in the glass a fluorescence which is the same as that of the rare earth itself. Another explanation is the crystalline structure of the glass, and this receives support from the work of M. Curie,[20] and of R. Thomaschek and O. Deutschbein.[26]

Experiments with glasses of the type $2B_2O_3,3ZnO$ showed that phosphorescence resulted when the glass was crystalline but not when it was in the vitreous state, and this difference is attributed to the interionic forces in the crystal lattice. The work of Thomaschek and Deutschbein indicated distinct differences between the glassy liquid and crystalline states of glass. The intensity and duration of this phosphorescence was shown to be influenced by heating at 800° to 1000° C. and by the addition of other elements, such as manganese, lead and bismuth. E. Rexer,[24] too, has shown that glasses of the type $Na_2O,2SiO_2$ in the crystalline state have a greatly increased phosphorescence and fluorescence on exposure to ultra-violet light, X-rays or cathode rays as compared with the same non-crystalline glasses. The position of the bands of the fluorescent light produced on excitation by X-rays can, in fact, be used to follow the molecular structure of the glass. It therefore seems that crystalline structure and chemical composition may both play some part in accounting for the effects observed. The fluorescence which some glasses acquire after exposure to X-rays or to ultra-violet light may provide a further means of identification.[13, 18]

Applications to legal work of this characteristic fluorescence of glass are described on page 233.

REFERENCES.

1 A. Guttmann, *Stahl und Eisen*, 1926, **46**, No. 42.
2 R. Grün and G. Kunze, *Zement*, 1926, **15**, 677.
3 V. Kubelka, *Chem. Listy*, 1929, **23**, 337.
4 A. Schmölzer, *Die Stein-Industrie*, 1927, vol. 12.
5 O. Krause, *Ber. Deut. Keram. Ges.*, 1926, **8**, 114.
6 H. B. Milner, *Quartz Lamp*, 1933, **2**, 4.
7 F. Mach and P. Lederle, *Phosphorsäur.*, 1932, **2**, 623.
8 B. E. Cohn, *Jour. Amer. Chem. Soc.*, 1933, **55**, 953.
9 W. B. Lester, *Glass Ind.*, 1931, **12**, 83.
10 M. Guillot, *Compt. rend.*, 1934, **198**, 2093.
11 O. Krause, *Keramos*, 1930, **9**, 791 ; *Trans. Ceramic Soc.*, 1930, **29**, 146.
12 H. Kersten and C. H. Dwight, *Jour. Opt. Soc. Amer.*, 1934, **24**, 285.
13 M., M. and H. Fischer, B.P. 415,536, 9/5/33 ; 421,142, 19/12/33.
14 H. Carlsohn and W. Voigt, *Chem. Fabrik.*, 5/3/35, p. 126.
15 W. Weyl and E. Thümen, *Sprechsaal Keram.*, 1934, **67**, 95.
16 D. M. Wilson, *Contract. Journ.*, *Suppl.*, 1/5/35.
17 J. Grant, *ibid.*, 1935, **113**, 504.
18 F. Eckert and K. Schmidt, *Glastechn. Ber.*, 1932, **10**, 80.
19 J. Smiles and H. Wrighton, *Proc. Royal Soc.*, 1937, **158A**, 671.
20 M. Curie, *Compt. rend.*, 1936, **203**, 996 ; 1937, **204**, 352 ; *Trans. Faraday Soc.*, 1938, **35**, 114.

[21] O. Deutschbein, *Z. Physik.*, 1936, **102**, 772.
[22] N. Tschernjavsky, *Cement* (U.S.S.R.), 1937, **11**, 35.
[23] C. Philipp (Private Communication, 24th April, 1938).
[24] E. Rexer, *Glastechn. Ber.*, 1938, **16**, 90.
[25] P. Gilard, L. Dubrul, F. Jamar, and D. Crespin, *Verre Silic. Ind.*, 1937, 8, 414; 1938, **9**, 253, 266; *Glass Ind.*, 1938, **19**, 99.
[26] R. Thomaschek and O. Deutschbein, *Glastech. Ber.*, 1938, **16**, 155; *Trans. Faraday Soc.*, 1939, **35**, 69.
[27] F. F. Lucas, *New Internat. Assoc. Testing Mat.*, 1931, Sept.

ADDITIONAL REFERENCES.

R. Mitsche, *Iron and Steel Inst. Rept.*, 1936, May (Applications to metals).

J. Grant, *Canadian Chem. and Met.*, 1936, **20**, 69 (Testing engineering materials).

J. A. Radley, *Electrical Times*, 1936, Feb. 15 (Testing electrical materials).

F. W. Martin, *Brit. Med. J.*, 1934, Nov. 24. (Identification of glasses).

J. Grant, *Glass*, 1935, **12**, 520; *Glass Ind.*, 1938, **19**, 387 (General).

B. E. Cohn and S. C. Lind, *J. Phys. Chem.*, 1938, **42**, 441 (Thermoluminescence of zinc borate glasses exposed to radium and excited by ultra-violet light).

D. A. Jones, *Glass*, 1936, **13**, 298 (Apparatus for testing glass in ultra-violet light).

S. S. Bhatnagar, *Nature*, 1939, **143**, 599 (Investigation of the valency of manganese in glasses).

CHAPTER V.

DRUGS.

For some time past workers on the Continent have examined the fluorescence in ultra-violet light of pharmaceutical preparations and drugs ; thus H. Neugebauer [46] has published a brochure on the capillary method for pharmaceutical work, and K. Göllner [1] has even classified drugs according as they are indifferent, fluorescent or phosphorescent. It is only recently, however, that English workers have turned their attention in this direction. Three papers by G. E. Trease,[2–4] and others by J. Grant [5] and J. A. Radley,[78] indicate some of the uses of the lamp in pharmaceutical laboratories, but these are only of a general nature.[64] A. D. Powell and Droop Richmond have examined olive oils, and another paper by J. Grant [6] on the titration of quinine is referred to on page 314.

A test for the effect of tropical heat on *ether for anæsthetics* is described by S. G. Liversedge,[76] who exposed the sample 7 inches from a source of ultra-violet light for various periods of time. He was then able to obtain a correlation between this test and the usual heat test (exposure at 120° F. for varying periods of time).

Drugs in powder form or in simple solution are examined in non-fluorescent containers under the fluorescence-microscope (see p. 78). No fluorescence is shown by the majority of alkaloids in solution, but those which fluoresce in neutral solution usually show a decrease in intensity on addition of alkali and an increase when acid is added. When a solution contains more than one compound the capillary strip method is of great value, and may often be used in the presence of other constituents, even if these form their own characteristic zones. Amounts of an alkaloid, too small to give a precipitate with Mayer's reagent, often form fluorescent zones ; *e.g.*, a blue zone is obtained with only 0·003 per cent. of morphine. It must be pointed out at

this stage that storage is liable to bring about changes in the reactions under the lamp, and this is particularly noticeable with the capillary strip method which is so delicate that small traces of compounds formed in this way may form fresh zones or modify the colour of those normally obtained.

Marcan [73] reports a method which he applied to the examination of 2 c.c. of a mixture of castor and chenopodium oils (in connection with a death arising from an inaccurately dispensed prescription); capillary tubes containing the specimen and known mixtures of the two oils were compared, and the former was found to contain an excessive quantity of chenopodium oil.

Alkaloids.—The fluorescence of quinine sulphate was first noted by J. Herschel in 1845, and the early investigations on alkaloids and some organic poisons were carried out in 1916 by R. Heller,[7] who used a luminescence microscope, and later by B. Jonsson.[43] The former divided such substances into three classes according as they fluoresced strongly, moderately or weakly. Table 7 includes some of his results, the plus and minus signs indicating a high and low intensity respectively, in the class concerned. It is interesting to note that the fluorescence of fluorescein solutions is inhibited by the addition of certain alkaloids, but A. Achard and his co-workers [79, 107] find that geno-alkaloids are without this effect (see p. 384). The diminution in intensity appears to be parallel with the reduction in toxicity and the disappearance of antioxidant properties of the drug.

P. W. Danckwortt and E. Pfau [8] have also investigated a number of drugs, and their method of examination sets the use of ultra-violet light for this purpose on a surer basis. They examined the strips obtained by capillary analysis (see p. 58) under the lamp and found that distinct bands of colour were visible due to the distribution of the various components of the preparations examined, the fluorescence being relatively brighter than that given by the drug in the solid state. They also tested a number of drugs in the dry solid state, without previous purification. A large number were found to fluoresce under the lamp in a marked manner. Quinine tannate and ferro-citrate, strychnine, brucine, daturine, and cocaine and its salts gave only a weak blue shimmer or practically no fluorescence, results more or less in accordance with those of Heller, and Tables 8 and 9 below indicate some of the fluorescence colours for comparison,

TABLE 7.

INTENSITY 1.

Aconitine.	White, with bluish tinge (—).
Berberine.	Bright yellow.
Caffeine.	Bluish-white (—).
Chitenine.	Blue-violet.
Cocaine.	White, with a bluish tinge (—); green-blue in warm H_2SO_4 containing resorcinol.[42]
Colchicine.	Light greenish-white (—).
Delphinine.	Greenish-white (—).
Pyramidon.	Bright blue (—).
Theobromine.	Bright blue (—).
Solanidine.	Green.[74]
Solanine.	Blue.[74]
Tropa-cocaine.	Bluish-white (—); violet-red in warm H_2SO_4 containing resorcinol.[42]

INTENSITY 2.

Aloin.	Reddish-yellow.
Apomorphine.	Bright blue.
Apoquinine.	Violet.
Atropine.	Bluish-white (+).
Emetine.	Yellow.
Homotropine.	White.
Hydrastine.	Bluish-violet (+).
Isoquinine.	Violet.
Narceine.	Bright green, with bluish tinge.
Physostygmine.	Bluish-violet.
Pilocarpine.	Dull bluish-white (—).
Quinine.	Pale blue.
Strychnine.	Greyish-blue (—).
Veratrine.	Bluish-white (+).

INTENSITY 3.

Anemonine.	Yellowish-grey.
Antipyrine.	Greyish-green (—).
Brucine.	Greyish-blue (+).
Cantharidine.	Yellowish-grey.
Cinchonidine.	Greyish-violet.
Cinchonine.	Grey, with a reddish tinge.
Codeine.	Grey.
Daturine.	Bluish-grey.
Ergotine.	Brownish-yellow.
Eucaine.	Grey (—).
Heroin.	Yellowish-grey.
Hordenine.	Green.[74]
Morphine.	Blue, with a violet tinge.
Narcotine.	Greyish-green, with a bluish tinge (+).
Papaverine.	Bluish-violet.
Picrotoxine.	Greyish-yellow.
Pilocarpine.	Greyish-brown.
Quinotoxine.	Yellow-white.
Thebaine.	Bluish-grey.
Veronal.	Yellowish-grey.

For general pharmaceutical work the above investigators [80] recommend spotting solutions or tinctures on filter paper, and suggest that the fluorescence may be developed or modified by allowing a spot of a reagent nearby to run into the first. The reagents suggested are: ten per cent. solutions of sodium hydroxide, hydrochloric acid, borax, aluminium sulphate, potassium cyanide and ammonia. The fluorescence colours so developed are best seen after the paper has been dried, and they are quite stable for long periods. These workers give the fluorescence reactions of many drugs tested by this method and consider that the method is particularly adaptable to the detection of aloin by borax and *vice versa*; of gambier by sodium hydroxide; of Persian ammoniacum by sodium hydroxide and borax; and of aluminium by morin. The test for aloin is diagnostic for this drug even in the presence of other emodin-containing materials. In the case of aluminium, the paper used must be free from traces of aluminium salts. K. Szahlender [103] considers that, contrary to general acceptance, Persian ammoniacum contains umbelliferone as shown by the fluorescence under the lamp.

J. Khouri [81] finds that hashish gives a brown to mahogany-coloured fluorescence under the lamp when examined in fragments, but that if finely-powdered this fluorescence disappears. An extract of hashish in light petroleum has a green fluorescence which disappears when exposed to air for some time, but which is, however, stable to heating at 100° C. for 30 minutes. Khouri uses the fluorescence obtained by extracting with various solvents to distinguish hashish from the few adulterants which yield fluorescent extracts themselves (*cf.* p. 51).

When extracted with ether hashish gives a green fluorescence due to the presence of cannabinol, and J. Khouri [106] considers this a more reliable test than the method of detection used by Beam, which is not specific for hashish. The presence of oil of cloves or of ginger masks the fluorescence. As little as 0·1 mgrm. of nicotine can be detected [82] in solution by its deep azure-blue fluorescence, and although technical nicotine can be detected in the same concentration the fluorescence is sometimes more reddish than that of the pure compound. The fluorescence is not affected by alkalis, but hydrochloric acid changes the tone a little towards green. The fluorescence of quinine sulphate at several *p*H values has also been studied by A. Boutaric

and J. Bouchard,[85] and M. Prost [86] has shown that short-wave radiation is emitted by the surface layers of quinine sulphate during hydration or dehydration, that emitted during the former being 6 to 8 times the greater.

The thalleioquinine reaction has been known since 1862, but although the blue fluorescence observed by Gremsa and Halberkann in 1921 has never been employed as a test, M. Haitinger [87] finds that when bromine water is added to a solution of quinine the fluorescence of the quinine appears lighter, and further addition causes it to disappear. When the extinction commences ammonia is added, and a yellowish-green fluorescence is produced. By means of the spot test, it is possible to detect so little as 0·4 γ of quinine at a dilution of 1 in 500,000 by this method. Quinidine gives the reaction, but alkaloids of the other groups do not.

A delicate method for estimating quinine in blood has been elaborated by F. J. Kaiser [114] who adds sodium nitrate to the sample to prevent coagulation, and then heats with sodium hydroxide and extracts with chloroform. The chloroform is evaporated, ether and sulphuric acid are added, and after removing the fatty matter present a clear solution of quinine sulphate results. This is compared in an apparatus described on page 63, and in this manner 0·5 γ of quinine may be estimated.

Bayle and Fabre [15-18] have examined a number of pure drugs of the *isoquinoline and tetrahydro isoquinoline groups* in the solid state, and have measured the fluorescence photometrically. They compared the total fluorescence-intensity with that of sodium salicylate, which they fixed arbitrarily at 20. Coumarin and its derivatives fluoresce weakly, æsculin being the strongest with a violet-coloured fluorescence of intensity 9. It may be noted here that the fluorescence of æsculin in dilute aqueous solution decreases when it is exposed to ultra-violet light, and this decay then continues, even in the dark.[19] Novocaine hydrochloride has a fairly strong fluorescence-intensity (32 on Bayle and Fabre's scale). Some isoquinoline derivatives are included in the results shown in Table 8. N. L. Allport and S. K. Crews [109] point out that in aqueous solution ergometrine shows a blue fluorescence more brilliant than that of ergotoxine.

Hydrastine sulphate shows no fluorescence when freshly prepared, but a fluorescence is slowly developed in the cold on

TABLE 8.

	Colour of Fluorescence.	Intensity.
Cotarnine	Nil	0
Cotarnine hydrochloride	Yellow	35
Hydrastinine	Nil	0
Hydrastinine hydrochloride	—	4
Hydrastine	Green	55
Hydrastine salicylate	Greenish-white	55
Hydrastine hydrochloride	Blue	36
Narceine	Violet-blue	7
Narcotine	Violet	—
Papaverine	Bluish-white	6
Potassium opianate	Blue	5
Opianic acid	Pale blue	10
Veratric acid	Pale blue	5

standing, and this is accelerated by heat, and is instantaneous if a few drops of hydrogen peroxide are added. Spectrophotometric curves of the drugs were also plotted.

Comparison of the above results with those of Danckwortt and Pfau (Table 9) indicates how the state of division of the drug

TABLE 9.

Aconitine.	Distinct light blue.
Apomorphine.	Deep blue.
Atropine sulphate.	Faint blue.
Berberine.	Distinct yellow.
Berberine hydrochloride.	Distinct yellow-green.
Cinchonine	Light bluish.
Cinchonine sulphate.	Distinct clear white.
Codeine.	Light clear yellow.
Colchicine.	Distinct yellow-green.
Emetine.	Distinct yellowish-red.
Hydrastine.	Distinct light green.
Morphine	Pronounced light green.
Narceine.	Pronounced yellow-green.
Narcotine.	Light green.
Papaverine.	Pale light yellow.
Pilocarpine.	White.
Piperine.	Pronounced light yellow.
Quinine hydrochloride.	Light blue.
Quinine sulphate.	Pronounced light blue.
Solanine.	Faint blue.
Thebaine.	Reddish-yellow.
Veratrine sulphate.	Marked light blue.
Yohimbine hydrochloride.	Deep yellowish-green.

affects its fluorescence colour, a conclusion which is of fairly general application.

Modifications of this method have also been employed by C. A. Rojahn [9] and Rapp,[10] and A. Andant [11-14] has investigated the fluorescence of alkaloids spectroscopically in light of wave-lengths 3650, 3130 and 2557 Å., and of those examined strychnine and brucine gave no characteristic spectra. Isomeric alkaloids give similar spectra, and introduction of CH_3– or –OCH_3 groups increases the fluorescence. The value of this method depends mainly on the facts that the substance is neither altered in any way nor used up. In later work shorter wave-lengths were used, and it was shown that alkaloids with absorption bands in the ultra-violet region become strongly fluorescent only when irradiated by light of very short wave-length (see also Dhéré [72]).

In fluorescence analysis, differences in concentration are also liable to influence the colour, as is shown by the work of H. Neugebauer [20] who has studied the colours of the fluorescence obtained with various drugs for increasing concentrations under certain specified conditions. The detection of alkaloids in human and other milk, in urine and in other body fluids, is discussed on pages 234 and 254.

L. Ekkert [51] has worked on the detection of antifebrin and phenacetin, and aspirin has been examined by H. Valentin [52] and by A. Edwardsson,[53] who was able to determine it volumetrically. P. Antonio [88] notes that the fluorescence colours of urea and pyrazole derivatives are mainly blue to violet, and he has tabulated his results both for the compounds themselves and for mixtures of them in therapeutic preparations.

E. H. Maechlin [115] points out, that in some preparations phenolphthalein is often accompanied by certain laxative bodies such as aloes, cascara, rhubarb, senna or frangula which contain polyhydroxyanthraquinone compounds (see p. 108), thus leading to difficulties owing to their similarity in chemical behaviour. A number of these anthraquinone derivatives give reddish tones in alkaline solutions, which can mask or simulate the presence of small amounts of phenolphthalein. To overcome this Maechlin treats the substance in potassium hydroxide solution with hydrogen peroxide, and so obtains phthalic acid if phenolphthalein is present. This is condensed by heating with resorcinol in the

absence of a condensing agent, when fluorescein is produced and gives an intense fluorescence in alkaline solution; Cascara sagrada tablets containing succinic or oxalic acid gave negative results. This method allows the detection of 5 to 10 γ of phenolphthalein.

J. R. Nicholls [47] has determined *quinine* by direct colorimetric comparison of the fluorescence at various concentrations; a sample was shown to contain 0·205 to 0·215 grm. of quinine, whilst chemical analyses gave a figure of 0·21 grm. and the prescription required less than 0·17 grm. per c.c. This worker used the same amount of sulphuric acid in all of his comparison samples. E. Canals and P. Peyrot,[48, 77] in their study of the molecular diffusion of light in fluorescent liquids, used quinine in sulphuric acid solutions; they found that the specific fluorescence is constant and the proportionality is such that on sound theoretical considerations the method may be used for the determination of small amounts (*e.g.*, $0{\cdot}4 \times 10^{-9}$ grm. per c.c.) of quinine [84] (see also p. 256).

E. Milanesi [49] has found that there is a close parallelism between the fluorescence of quinine salts and their toxicity to protozoa, and these observations are of interest also from the point of view that certain fluorescent compounds sensitise the response of bacteria to the action of ultra-violet rays (see Waters, p. 378). Another interesting observation is that of S. Soule,[50] namely, that valerianate of quinine shows triboluminescence, whereas the other salts of quinine he tested do not.

The titrimetric determination of quinine is described on page 314, and further work on the fluorescence of *quinine*, its salts and derivatives, has been carried out by O. Marschall,[21] Wunderling and H. Fischer,[22] in the two latter cases more from the point of view of the uses of the method in legal medicine. With a few unimportant exceptions all the alkaloids of the quinine family have a blue or violet fluorescence.[72, 119]

Specific reactions for alkaloids are as follows :—

Physostigmine (after Petit's reaction). Sulphuric acid and then ammonia are added, and the residue after evaporation is dissolved in alcohol and acetic acid is added; a red fluorescence (also produced by phthalic acid) results.

Veratrine (after Formanek's reaction). Addition of pure concentrated sulphuric acid produces an orange-red colour and a yellow-green fluorescence.

Codeine (after Heller's reaction) has a yellow fluorescence in the presence of picrolonic acid.

Morphine.—In C. C. Fulton's test,[83] a little of the alkaloid is heated at 40° C. for 7 to 8 minutes with 0·5 c.c. of concentrated sulphuric acid, after which it is diluted with 5 c.c: of water and, without cooling, 6 c.c. of concentrated ammonia are added. The solution turns brown, and gradually a beautiful purple fluorescence develops. At room temperature the colour requires 2 to 3 hours to develop, but heating at 40° to 60° C. hastens the development. The optimum amount of morphine is 0·5 mgrm. and the limit of sensitivness is about 0·025 mgrm. Pseudomorphine and codeine do not give the test which is, however, given by heroin.

Miscellaneous.—*Ouabain* in arrow poisons; W. D. Raymond [108] finds that this may be distinguished from strophanthin by adding to the suspected substance 0·3 c.c. of a reagent made by dissolving a few crystals of naphthoresorcinol in concentrated hydrochloric acid, and heating to 50° C. on the water bath, and then adding 0·3 c.c. of amyl alcohol. A greenish fluorescence in the alcohol layer shows the presence of ouabain. Small quantities of *boldine*, from the South American drug boldo, give a milky blue fluorescence on dipping a filter paper into a solution of the hydrochloride, and spotting the paper with sodium hydroxide solution.[113] *Podophyllin*, which is sometimes added, may be detected by its strong green fluorescence.

F. Hoeke [110] distinguishes between *bourbonal* and *coumarin* by heating with resorcinol and concentrated sulphuric acid, adding excess sodium hydroxide and diluting, when a yellow-green fluorescence is given by bourbonal and a green blue fluorescence by coumarin, (see p. 294). The same process carried out with saccharin has been found by H. J. Vlezenbeek [111] to result in an orange fluorescence, 1 mgrm. of saccharin in 5 to 6 litres of water being detectable. Positive reactions are also obtained with citrophen, metol, salophen, methylacetin, *p*-anisidine, *p*-phenetidine, phenocoll hydrochloride, dulcin, lactophenin (green fluorescence), cryofin, glycine and *p*-acetamino-phenylallyl ether. All the above compounds give reddish coloured solutions with a transient reddish-brown fluorescence. The colour of the solution changes to purple overnight, and the fluorescence disappears. Aspirin, caffeine, pyramidone and codeine hydrochloride

show a green fluorescence on the addition of alkali, but the solutions do not change colour on oxidation. Sodium-, neo-, or myo-salvarsan gives a light red solution with a green fluorescence, which is unchanged by the addition of oxidising agents.

M. Pesez [112] finds that several *barbiturates*, when heated with concentrated sulphuric acid and a few drops of 40 per cent. formaldehyde on the water-bath, give an orange-red colour with an intense green fluorescence. Dilution of the cooled liquid destroys the colour, but the fluorescence remains. Dial, Numal and Sandoptal were found to react also, but Phanodorm gives a yellow colour which turns orange, and on dilution, brown, with a green fluorescence.

A number of non-alkaloidal drugs and herbs, etc., were also investigated by Danckwortt and Pfau,[8, 41] both in the powdered form and by the method of capillary analysis, and some of the results for the solids are given below :—

Cortex Granati, light patches on the outside ; *Cortex Condurango*, deep yellow patches ; *Cortex Cinchonae*, deep yellow patches side by side with some light blue patches. When the inside surface is touched with acid a light blue patch of colour appears immediately. *Cinchona bark* when sifted into water gives a liquid which shows no fluorescence under the lamp, but on addition of acid the whole liquid assumes a light blue fluorescence.

With *Radix Ipecacuanhae*, all places where the wood is visible, owing to the removal of the bark, fluoresce brightly, and addition of acid to these spots causes the appearance of a bright blue fluorescence. *Radix Levistici*, which contains umbelliferone, turns blue on addition of acid. A fresh section of *Radix Colombo* is intensely yellow, whilst the cambian ring and the vascular bundles appear dark green. *Radix Rhei* shows a brightly-luminous grain, and *Rhizoma Hydrastis* a beautiful golden shimmer wherever a broken surface is exposed. *Rhizoma Veratri* shows intense light blue spots, and in the cross-section of *Semen Arecae* the endosperm may be picked out by means of its beautiful light blue-coloured fluorescence. H. Eschenbrenner [54] detects *Radix Ipecacuanhae* in the following manner : The sample is treated with ammonia and is then shaken with chloroform, the extract being then separated and covered with a layer of water of equal depth. A milky yellowish fluorescence is observed in the chloroform

TABLE 10.

Drug.	Alkaline Solution.	Acid Solution.
Catechu.	Reddish-grey. Yellowish zone, shading to brownish-violet. Brown edge.	Whitish-yellow, blending to bluish. Edge whitish-violet.
Cortex Cascarillae.	Nearly colourless, with whitish edge.	Same, with violet edge.
Cortex Cinnamoni.	Dirty grey, with light edge.	Same, with pale violet edge.
Cortex Quillaiae.	Dark and bright grey zones, with white edge.	Uniform, bright, yellow-grey with violet edge.
Cortex Rhamni Purshiani.	Pale yellowish-red, lighter yellow at edge.	Same, with light violet edge.
Cortex Viburni.	Bluish-violet. Lighter whitish edge.	Same, brighter luminous violet edge.
Flores Althaeae.	Light greenish-yellow, then grey. Light violet-brown edge.	Bright grey zone, shading to a bright greenish edge.
Flores Arnica.	Almost uniformly violet. Yellow-green edge.	Narrow bright violet zones. Violet edge.
Flores Caryophylli.	Black to dark grey. Lighter edge.	Yellowish tinge. Lighter edge.
Flores Chamomillae Romanae albis.	Bright deep grey to bright violet. Pale violet edge.	Dark narrow zones and bright light-violet zone. Pale violet edge.
Flores Papaveris Rhoeados.	Yellowish, then dirty grey-violet. Darker edge.	Dark yellow, dark grey-violet, bright grey-yellow. Violet edge.
Flores Rosae. (Gallicae).	Dark then bright yellow-brown. No border zone.	Very dark brown, becoming lighter. Whitish luminous edge.
Folia Hamamelidis.	Light yellowish-grey. Yellow edge.	Yellowish, dark grey, and weak light violet zones. Violet edge.
Folia Hyoscyami.	Light grey to light green-violet zone. Dark edge.	Same. Luminous edge.
Folia Sennae.	Reddish-grey. Dark and light grey zones. Luminous light yellow edge.	Yellow to bright grey. Narrow light violet and grey zones. Light yellow edge.
Folia Stramonii.	Weak violet, then bright greenish-grey. Darker edge.	The same.
Folia Uvae Ursi.	Two grey zones. Yellow edge.	Same, with pale lilac edge.
Folliculi Sennae.	Reddish-brown. Yellow edge.	Yellowish-grey. Yellow edge.
Fructus Anisi.	Many light grey zones. Lighter edge.	The same.
Fructus Cardamomi.	Bright, light grey edge.	Yellow to light grey. Brighter edge.
Fructus Cassiae.	Light grey-brown. Yellow edge.	Grey-violet to light grey zones. Whitish edge.
Radix Liquiritiae.	Luminous bright yellow-green, and bright grey zones. White edge.	The same, but brighter.
Radix Pyrethri.	Colourless. Whitish edge.	Weak light violet. Violet edge.

layer, and on the addition of concentrated hydrochloric acid and shaking, the fluorescent compound is transferred to the water layer which assumes a strong bright blue fluorescence. M. Déribéré [89] finds that even dilute extracts of *Chelidonium majus* in alcohol or water show a characteristic golden-yellow fluorescence; 109 crude drugs of the National Formulary VI (America) have been examined by M. S. Dunn and W. H. Kimmer.[90]

P. Ernst and E. Jentschitsch [23] have examined a number of drugs and preparations used in pharmacy by means of the capillary method, and of the 132 samples which they examined, some of the more important are given in Table 10. They extracted 1 grm. of the drug with 100 c.c. of distilled water, and the strips of paper used were 30 cms. long and 2·5 cms. wide, the ends being dipped in the liquid to a depth of about 2 to 3 cms. and, after an hour, removed, dried and then examined under the lamp. To portions of the solutions were added 5 c.c. of acetic acid, or 5 c.c. of *N* ammonia.

Table 11 gives the appearance of the strips obtained with some of the less important drugs.

R. Wasicky [24, 25, 26] has investigated a large number of substances used in pharmacognosy under the luminescence microscope (see p. 78), and his work on the detection of cocoa husk in cocoa powder is described on page 165. *Gentian* powder shines with a white or light-blue colour, and adulteration with *Rumex* powder is detectable by the golden-yellow or dark-green fluorescence due to the latter.

In his investigation on the *Rhubarbs*, Wasicky treated them with a cold solution of borax in glycerin; in this way every cell containing the oxymethylanthraquinone glycoside appears with a beautiful green sheen. Rheum emodin and rhei are, however, insoluble in this solution. When the preparation is examined under the microscope it is found that the green sheen is actually yellow to reddish-yellow in colour, but that the application of heat develops the green colour. A bright greenish-white shimmer is obtained from the cells of fresh spring rhizome when they become filled with the borax solution, the green colour being enhanced by treatment with water although the shimmer disappears. Incidentally, this test has been found to be of general application, and C. A. Rojahn [117] records data for 270 drugs in acid and alkaline solutions.

Further work on rhubarbs was carried out by P. Bretin and A. Leulier [27] (who also examined the fluorescence of aloes, guaiacum, colophonium, turpentine and other drugs), although their work on these substances is not exhaustive. J. Maheu [28] found that true official rhubarbs give a brownish-red fluorescence whilst *Rheum Compactum*, *Undulatum*, *Ribes* and *Rhaponticum* show a violet fluorescence visible throughout the entire rhizome whether in the form of powder or tincture. *R. emodi* has a brownish-red fluorescence identical with that of the true official rhubarbs, and Austrian and French rhubarbs both give violet colours. With mixtures of rhubarb and rhaponticum, a violet fluorescence is observed, which deepens in colour as the percentage of the latter decreases, 10 per cent. being easily detectable. Maheu also describes a micro-method applicable to so little as 1 per cent. of rhaponticum. Photograph No. 2 (facing p. 400) shows five authentic types of rhubarb examined by J. Grant and H. Procter-Smith.

S. K. Crews [91, 92] has examined many samples of rhubarbs of various species and has evolved a very easy but delicate test for the detection of *Rhaponticum*. A tube is filled with best quality cotton wool which has been extracted with ether to remove any fats or oils, and the tincture is poured through. After the liquid has percolated through, the cotton wool is washed until it retains only a faint yellow colour. Under the lamp the cotton wool will appear a bright blue if *Rhaponticum* is present even in small quantities (*e.g.*, 0·01 per cent.). S. K. Crews has separated the fluorescent principle of *Rhaponticum*, which appears to be concentrated in the region of the cambium layer,[93] and he finds it to be rhaponticin.

Tinctures.—With tinctures (see below) similar colours are observed.[44] An alcoholic extract of rhaponticum has a milky-white fluorescence, and with official rhubarbs an orange-brown fluorescence is observed. T. E. Wallis and E. R. Withell [55] find that additions of 10 per cent. of rhapontic to Chinese rhubarb changes the fluorescence of the latter from a brown to a purple, and if a hand-lens is used to observe the fluorescent purple spots, so little as 5 per cent. is detectable. When only 1 per cent. of rhapontic is suspected to be present, they use 2 per cent. extracts prepared by macerating the powdered sample with alcohol for 18 hours in the dark, followed by filtration. Strips of

TABLE 11.

Cortex Condurango.	Pale violet, with light edge.	Same, with violet edge.
Cortex Frangulae.	Bright orange-yellow, light green and dark violet zones. Yellow edge.	Same, with lighter green zone and bright violet edge.
Cortex Quercus.	Dirty grey and whiter edge.	Same, only bright violet edge.
Flores Chamomillae, Vulgaris.	Narrow light yellow and dark violet zones, with narrow white edge.	Green-yellow and narrow dark brown zones. Bright yellow, lighter edge.
Flores Croci.	Dark brown to yellow. Yellow edge.	Same, with violet edge.
Flores Koso.	Dark, then bright grey zones becoming lighter. Yellow edge.	Dark brown, then weaker. Violet edge.
Flores Lavandulae.	Bright green, with a dark brown edge.	Dark violet, then a very dark and a light zone. Pale luminous edge.
Folia Jaborandi.	Almost colourless. Lighter edge.	The same.
Folia Rosmarini.	Light grey and almost colourless narrow edge.	Dark grey to weak violet. Light narrow edge.
Foliae Theae, Ceylon	A bright and a dark brown and then a narrow grey zone. Yellowish edge.	Light, then a narrow dark brown zone changing to light grey-violet. Violet edge.
Fructus Anisi Stellati.	Light grey. Bright whitish edge.	Light yellow to light grey. Violet edge.
Fructus Capsisi anni.	Very bright, with brighter white edge.	The same.
Fructus Carvi.	Light grey, with two grey zones. Brighter edge.	Light yellow and light grey zones. Brighter edge.
Fructus Juniperi.	Light grey-brown, with whiter edge.	Narrow dark brown and light grey zones.
Fructus Lauri.	Light grey. White edge.	Dark, then light grey, with a pale blue edge.
Fructus Piperis.	Bright blue-green and nearly colourless zones. Greenish edge.	Same, with a bright violet edge.
Fructus Vanillae.	Light yellow to green-violet. White edge.	Light yellow to grey-violet. White edge.
Herba Cannabis.	Almost colourless. Green-brown edge.	Dirty green, then light grey. Whiter edge.
Herba Conii.	Pale and bright light violet zones. Lighter edge.	The same.
Herba Convallariae (maialis).	Weak yellow, then light green. Brown edge.	Yellow, then bright pale violet, with a greenish-violet edge.
Herba Sabinae.	Light grey-violet edge.	The same.
Herba Sassafrass.	Greenish-grey. Whiter edge.	Weak violet and then greenish-grey zones. Whiter edge.

TABLE 11—*Continued.*

Radix Althaeae.	Nearly colourless. Whiter edge.	The same.
Radix Iridis.	Yellowish-grey. Whitish edge.	Bright grey, then dark zones, the latter decreasing in intensity. Violet edge.
Radix Liquiritiae. (Russ).	Dull yellow-green and grey zones.	The same, but brighter.
Radix Rhei (Austr.).	Luminous dark violet, then dark brownish-yellow edge.	The same, but duller.
Radix Rhei.	Dark red, with a darker edge.	Red to violet zone Whitish edge.
Fructus Colocynthidis	Yellowish to grey. White edge.	The same.
Fructus Coriandri.	Light grey. Whiter edge.	Similar.
Fructus Cubebae.	The same.	The same.
Fructus Tamarindi.	The same.	Light yellow and dark brown zones. Light greenish-violet edge.
Herba Lobeliae.	Light grey. Yellow edge.	Light grey-violet edge.
Lignum Guaiaci.	Grey to almost colourless. Brilliant edge.	Lilac, becoming lighter at the edge.
Lignum Haematoxyli.	Light yellow to light green. Bright violet edge.	The same.
Lignum Quassiae.	Whitish to bright lilac, and bright light grey edge.	The same, but a narrower lilac zone.
Lignum Santali.	Weak lilac to grey. Whiter edge.	Light violet to light grey. Darker violet edge.
Radix Arnicae.	Weak violet. Darker edge.	Bright light violet. Darker violet edge.
Radix Belladonnae.	Light to dark violet. Whiter edge.	The same, with bright violet edge.
Radix Calumbae.	Bright yellow and narrow dark brown zones. Narrow violet edge.	Same.
Radix Filixmas.	Dark yellow to grey. Whitish edge.	Yellowish to light grey. Whitish edge.
Radix Gentianae.	Yellowish to bright grey. Yellow edge.	Same, violet edge.
Radix Hydrastidis. (Canadensis).	Bright yellow luminous and blue zones.	Bright light yellow, bright blue, and also a narrow bright blue zone.
Radix Ipecacuanha.	Bright and dark grey zones. White edge.	Bright yellow and bright blue and grey zones. Violet edge.
Radix Jalapae.	White violet. Bright edge.	Bright grey, luminous bright violet and bright grey zones. Bright violet edge.

non-fluorescent absorbent paper are then soaked in the tinctures, dried in the dark, and examined in filtered ultra-violet light, when 2 per cent. of rhapontic rhubarb can be detected by the purple colour it imparts to the brown fluorescence of Chinese rhubarb. This is gradually replaced by a yellow colour on exposure to daylight, ultra-violet light or to a temperature of 70° C., or if the operations are not carried out in the dark.

They find—

1. That on exposure of the strips to ultra-violet light the violet fluorescence fades, but the yellow fluorescence does not.
2. Daylight gives similar results but the process of fading takes longer.
3. Exposure of the strips to a temperature of 70° C. seriously diminishes the fluorescence.
4. Exposure of the powdered drug under the lamp for 40 minutes destroys the fluorescence at the surface; the same occurs more slowly in daylight.
5. That the filter-paper used should preferably be made from wood pulp, as cotton papers show " fluorescence with a strong violet tint " (see, however, Paper, p. 335).

That the importance and value of the method is being increasingly recognised is shown by the fact that the British Pharmaceutical Research List [56] includes it as a suggested subject for research.

The tinctures described in the German Pharmacopœia have been examined by F. Scheermesser,[57] and those of the Hungarian Pharmacopœia by I. Temesvary [58]; further work on tinctures and alkaloidal solutions is due to C. A. Rojahn and co-workers.[59, 96, 117] L. Zechner and R. Gager [94] point out that aqueous or alcoholic extracts of the same drug give the same type of capillary strip. They use the capillary method for the detection of tincture of arnica.

J. Deiniger [60] uses the capillary strip method for the examination of tinctures and classifies them as shown on opposite page.

Class 1. (One colour only.)

Tr. veratri.	Violet.
Ext. chinæ fl.	Brilliant violet.
Tr. strychni.	Blue.
Tr. strophanthi.	Bluish.
Tr. scillæ.	Very dark bluish.
Tr. capsici.	Very dark bluish.
Tr. rhiz. grami.	Dark blue.
Tr. calami.	Reddish-violet.
Tr. aconiti.	Dark blue.
Tr. benz.	Greyish-green.
Tr. catechu.	Dark brown.
Tr. myrrhæ.	Yellow.
Tr. gentianæ.	Yellow.
Tr. zingib.	Yellow.
Tr. ratan.	Red-brown.
Tr. aloes.	Brown.
Tr. aloes co.	Brown.
Ext. aurantii fl.	Bright brown.
Ext. frangulæ fl.	Red-brown.
Ext. hydrastis fl.	Clear yellow.

Class 2. (Two coloured zones.)

Tr. arnicæ.	Blue and pale blue.
Tr. absinth.	Greenish-grey and a blue zone.
Tr. chinæ spl.	Dark blue and red-brown zones.
Tr. chinæ co.	Blue and dark red-brown.
Tr. colchici.	Greenish-blue and brilliant blue zones.
Tr. colocy.	Blue and yellow zones.
Tr. ipecæ.	Blue and brilliant blue.
Tr. rhei vin.	Violet and brown.
Tr. tormentil.	Red and red-brown.
Tr. lobeliæ.	Blue and dark red-brown.
Tr. valerianæ.	Blue and red-brown.
Ext. thymi fl.	Yellow and brown.

Class 3. (Several colours with a characteristic zone.)

Tr. aurantii.	Red-yellow with a blue zone.
Tr. aromat.	Grey-green with a blue zone.
Tr. canthar.	Bright blue with a yellow zone.
Tr. digit.	Brown and violet with a red-brown zone.
Tr. gall.	Grey-green with a blue zone.
Tr. opii benz.	Dark blue, then light blue, and a yellow zone.
Tr. opii croc.	Brilliant brown with a green zone.
Tr. opii spl.	Green-yellow with a blue zone.
Tr. pimpinellæ.	White with a yellow zone.

D. R. McCullagh, C. H. A. Walton, and F. D. White [29] have examined the fluorescence of the roots of *Diervilla diervilla* and the leaves of *Symphoricarpos occidentalis* under the lamp, and

have shown that they contain the fluorescent glycosides fraxin and æsculin, respectively. Further work on pharmaceutical preparations has been carried out by Wöllmer,[30] by Günther[31] and by K. Göllner.[1] A. Kuhn and G. Schäfer[104] have examined homeopathic essences and tinctures of *Chimaphila umb.*, *Pirola unifl.* and *rotundif*, *Epigaea rep.*, *Gaultheria proc.* and a number of other drugs, and find arbutin, hydroquinone or ericolin as characteristic ingredients. A peculiarity in the capillary analysis of *Chimaphila umbellita* is the formation of yellow crystals of ursone in the middle zone. Some of the tinctures were evaluated by these workers by estimating their arbutin and hydroquinone contents. They also examined certain homeopathic preparations containing coumaric acid derivatives, *e.g.*, coumarin, umbelliferone, daphnin, fraxin and scopoline.

A number of natural *oils*, *balsams*, *resins and esters* have been examined by C. A. Rothenheim,[32, 19] in solutions in ether, ethyl acetate, ligroin, acetone, petroleum spirit, benzene, chloroform, methyl alcohol and water. It was found that the only fluorescence suitable as a test was obtained from aqueous preparations of Peru balsam, which gave a bright blue colour (see, however, p. 325).

The method was used with more success by A. Rosenfeld and S. Bolotnikov[33] to differentiate between preparations made from the American *Viburnum prunifolium* and the Russian *Viburnum opulus*. The former fluoresces with a reddish colour, whilst the latter appears bright green. Ergot fluoresces blue; convallamarin gives a green colour; digitoxin, sky-blue; digitalin, weak yellowish-green; digitonin, weak sky-blue; strophanthin, bright blue; scillotoxin, reddish-yellow.

The examination of pine needle extracts by the capillary method of Danckwortt and Pfau (p. 58) has been carried out by H. Eschenbrenner,[34] who concludes that the efficiency of the method compares favourably with that of other methods in general use. Pine needle extracts (as used in therapeutic baths) have an olive-green fluorescence, and adulteration with sulphite waste liquor (*cf.* p. 340) is indicated by a greyer shade, the intensity of which is proportional to the amount of sulphite liquor present.[118]

H. Neugebauer[35, 45] has examined hydrastis, *berberis vulgaris* and *aquafolium*, *sanguinaria*, *coccus cacti*, *piper methysticum*, *rheum*, *crocus* and *sinapis alba* by dropping a bead of the liquid or solution on a filter-paper, and examining the resulting rings

by a modification of Danckwortt's method. *Ginseng* appears green or, in exceptional cases, blue.

As mentioned on page 105, P. W. Danckwortt has used the method for the detection of *chlorophyll in pharmaceutical tinctures*, but as G. Zickgraf [36] points out, preparations containing chlorophyllin do not necessarily show the reactions characteristic of chlorophyll. In the preliminary qualitative analysis of complex preparations, the bright red fluorescence of chlorophyll is often of assistance for the detection of the presence of a drug derived from leaf tissue, and as indicated on page 301, it is possible to differentiate between chlorophyll prepared for medicinal use and the cheaper varieties which are used for colouring soaps and other products. Danckwortt and Pfau [37] shake the alcoholic tincture or extract with wet ether, when the chlorophyll passes into the ether. Alternatively, the capillary-strip method is employed, and the chlorophyll is extracted from the appropriate zone with ether. All the green parts of various plants contain chlorophyll which may be extracted in this manner. Red beech leaves and certain maple leaves do not, however, give the reaction.

F. Grégoire and J. Ripert [38] found that in the distillation of commercial orange-flower waters the fluorescence is concentrated mainly in the first fraction of the distillate, and that the intensity of this fluorescence decreases with age owing to the presence of fungi and to the influence of light. They therefore propose to use this method for the evaluation of commercial orange-flower waters, particularly with respect to their age.

Galenical preparations have been examined under the lamp by L. Zechner and F. Gstirner,[39] who obtained a series of fluid extracts and examined these in ordinary daylight and in filtered ultra-violet light. The reader is referred to the original papers for the tabulated results.

Ointments.—C. A. Rothenheim [61] has used the capillary strip method for examining ointments and ointment bases, but W. Peyer,[62] who has examined the drugs in the Supplementary German Pharmacopœia, extracts the drug in petroleum spirit and examines the fluorescence of the residue after evaporation. Ointments and pastes may also be extracted in duplicate with warm water, the two portions being made acid and alkaline (with dilute sulphuric acid and sodium hydroxide) respectively.

Quinine is often detectable by this method in the acidified portion, whilst in the alkaline portion the presence of salicylic acid, acetyl salicylic acid, or their salts is indicated by a strong violet fluorescence; alkaline solutions of urotropine, caffeine, theobromine, thiosinamine and piperazine show no fluorescence.[63]

Mineral greases are readily detected in ointments and *mineral oils* in other oils (see p. 191). E. J. Kocsis [95] points out that in the examination of ointments using the fluorescence microscope the colours observed show distinct variations in tone according as white or yellow vaseline is used as the base. A sample of oil supposed to be genuine seal oil gave an intensive blue fluorescence, showing the presence of a considerable amount of mineral oil, and chemical examination showed the presence of at least 40 per cent. It may not be out of place to mention here the fluorescence of *essential oils*, although this subject is dealt with more fully on page 302. Thus G. Rattray and G. R. Milne [64] mention that *Ol. Theobrom.* obtained by expression shows no fluorescence, whilst samples obtained by extraction have a characteristic fluorescence. One of us (J. A. R.[63]) has found that there are small but distinct differences between English, American and Japanese peppermint oils, but the determination of mixtures by direct observation is very uncertain. The sensitiveness of the B.P. test for differentiating between English and Japanese peppermint oils is greater in ultra-violet light than in daylight. Rattray and Milne [64] have also found that diethyl phthalate (which is now added to surgical spirit) fluoresces, and they mention the fact that the American Customs authorities use this to control the distribution of spirit for special purposes (*cf.* p. 224).

The fluorescence of *homeopathic triturates* under the lamp has been studied by A. Kuhn,[40] who also used Danckwortt's capillary method, and by H. Neugebauer.[35, 45] He made determinations of the homogeneity and particle size of triturates of uranium nitrate and mercurous chloride, and observes that the greater the degree of comminution the more marked is the fluorescence colour of the lactose present compared with that of the substance under examination. In this branch of the subject work has also been done by P. W. Danckwortt,[8, 41] who examined homeopathic preparations containing calomel, uranyl nitrate, salicylic acid, quinine sulphate and hydrastine in the proportions 1 in 10, 1 in 100, etc., up to 1 in 1,000,000. A mixture of 1 part

in 10 of calomel appears red, and on further dilution, turns violet. The 1 in 1,000,000 sample could not be differentiated from pure milk sugar.

Attempts to correlate the fluorescence with the pharmaceutical activity of various preparations of *zinc white* made by J. Eisenbrand and G. Siewart [65, 75] led to no definite conclusions. Fluid organotherapeutic preparations have been examined by F. Wischo,[66] and H. Neugebauer has studied homeopathic preparations containing gelsemium and aconitum,[67] ginseng [68] and other substances.[69-71, 102]

Although not strictly within the limits of this book it should be mentioned that several ointments have recently been introduced to prevent sunburn by absorbing the solar ultra-violet rays. According to B. Reichert and H. Böhme [97] in Engadina Sunburn the active agent appears to be β-umbelliferone-acetic acid, which has been recommended for this purpose by Mannich.[100] Amides or esters of naphthocoumarin carboxylic acid,[98] dibenzylidene-acetone [99] and *o*-amino benzoic acid [101] are also used.

Reference must be made to Chapters VI and XIV for further information dealing with the investigation of organic substances; and to Chapter XII for pharmaceutical substances of mineral origin (*e.g.*, chalks).

REFERENCES.

[1] K. Göllner, *Pharm. Monatsh.*, 1929, **10**, 206.
[2] G. E. Trease, *Pharm. Jour. and Pharmacist*, 1930, 162.
[3] G. E. Trease, *ibid.*, 1930, 187.
[4] G. E. Trease, *ibid.*, 1930, 264.
[5] J. Grant, *Chemist and Druggist*, 1932, **116**, 421.
[6] J. Grant, *Analyst*, 1931, **56**, 653.
[7] R. Heller, *Intl. Zeit. f. phys. chem. Biologie*, 1916, **2**, 397.
[8] P. W. Danckwortt and E. Pfau, *Arch. Pharm. u. Ber. Deut. pharm. Ges.*, 1927, **265**, 68; *Analyst*, 1927, **52**, 707.
[9] C. A. Rojahn, *Pharm. Ztg.*, 1929, **74**, 14.
[10] Rapp, *ibid.*, 1928, **73**, 1585.
[11] A. Andant, *Compt. rend.*, 1927, **185**, 713.
[12] A. Andant, *ibid.*, 1929, **189**, 98.
[13] A. Andant, *Bull. Sci. Pharm.*, 1930, **37**, 28.
[14] A. Andant, *Compt. rend.*, 1927, **184**, 1068.
[15] E. Bayle and R. Fabre, *ibid.*, 1924, **178**, 2181.
[16] E. Bayle and R. Fabre, *Jour. Pharm. et Chim.*, 1924, **29**, 535; 1925, **30**, 248.
[17] E. Bayle, R. Fabre and H. George, *Bull. Soc. Chim.*, 1925, **37**, 89.
[18] E. Bayle, R. Fabre and H. George, *Chim. et Ind.*, 1922, **17**, 179 (Chem. Abst., 1923, ii, 204).

[19] C. A. Rothenheim and L. Lettenmayer, *Pharm. Ztg.*, 1930, **75**, 862.
[20] H. Neugebauer, *Pharm. Zentr.*, 1929, **24**, 1511.
[21] O. Marschall, "Ueber Rotations- und Fluorescenzerscheinungen an China-Alkaloiden" (Diss, Jena, 1910).
[22] H. Fischer, "Die physikalische Chemie in der gerichtlichen Medizin und der Toxikologie mit spezieller Berücksichtigung der Spectrographie und der Fluorescenzmethoden" (A. Rudolf, Zurich), 1925.
[23] P. Ernst and E. Jentschitsch, *Pharm. Monatsh.*, 1929, **10**, 67.
[24] R. Wasicky, *Pharm. Post.*, 1913, **46**, 877.
[25] R. Wasicky, *Ber. Deut. botan. Ges.*, 1915, **33**, 37.
[26] R. Wasicky, "Fluorescenzerscheinungen. Handbuch der prakt. u. wiss. Pharmazie" (Urban and Schwarzenberg, Berlin), 1924.
[27] Ph. Bretin and A. Leulier, *Bull. Sci. Pharm.*, 1924, **31**, 360.
[28] J. Maheu, *ibid.*, 1928, **35**, 278 ; E. A. Lum, *Pharm. Jour.*, 1932, **75**, 571.
[29] D. R. McCullagh, C. H. A. Walton and F. D. White, *Trans. Roy. Soc. Canada (Biology Section* 5), 1929, **23**, 159.
[30] Wöllmer, *Jour. Pharm. Chim.*, 1927, **8**, 435.
[31] Günther, *Pharm. Monatsh.*, 1927, **8**, 27.
[32] C. A. Rothenheim, *Pharm. Ztg.*, 1929, **74**, 712.
[33] A. Rosenfeld and S. Bolotnikov, *Pharm. Jour.* (Russia), 1928, 31.
[34] H. Eschenbrenner, *Pharm. Presse. Wiss. prakt.*, 1930, 177.
[35] H. Neugebauer, *Apoth. Ztg.*, 1929, **44**, 1511 ; *Pharm. Ztg.*, 1932, **77**, 796, 1154 ; *Pharm. Weekblad*, 1933, **70**, 1221.
[36] G. Zickgraf, *ibid.*, 1930, **45**, 361.
[37] P. W. Danckwortt, *ibid.*, 1930, **45**, 207.
[38] F. Grégoire and J. Ripert, *Bull. Sci. Pharm.*, 1931, **38**, 209.
[39] L. Zechner and F. Gstirner, *Pharm. Monatsh.*, 1930, **11**, 6, 74, 221 ; 1931, **12**, 28.
[40] A. Kuhn, *Chem. Zentr.*, 1930, ii, 1742.
[41] P. W. Danckwortt, "Lumineszenz-Analyse im Filtrierten ultra-violetten Licht" (Leipzig, 1934).
[42] T. Wikander, *Pharm. Ztg.*, 1930, **76**, 1360.
[43] B. Jonsson, *Svensk. farm. Tidskr.*, 1931, **35**, 659 ; through *Chem., Zentr.*, 1932, i, 712.
[44] A. Castiglioni, *Ann. Falsif.*, 1933, **26**, 41.
[45] H. Neugebauer, *Pharm. Zentr.*, 1929, **24**, 1511 ; *Pharm. Ztg.*, 1930, **75**, 885 ; H. Schmidt-Hebbel, *Pharm. Zentr.*, 1932, **73**, 657.
[46] H. Neugebauer, "Die Kapillar-Lumineszenzanalyse im pharmazeutischen Laboratorium" (Leipzig : W. Schwabe, 1933).
[47] J. R. Nicholls, Private Comm., 6/6/34 ; *cf. Analyst*, 1934, **59**, 277.
[48] E. Canals and P. Peyrot, *Compt. rend.*, 1934, **198**, 746.
[49] E. Milanesi, *Arch. Farmacol. sperim.*, 1928, **46**, 29, 33.
[50] S. Soule, *Chem. Analyst*, 1929, **18**, 11.
[51] L. Ekkert, *Pharm. Zentralhalle*, 1930, **71**, 179.
[52] H. Valentin, *Apoth. Ztg.*, 1932, **47**, 381.
[53] A. Edwardsson, *Svensk. farmac. Tidschr.*, 1929, **33**, 557.
[54] H. Eschenbrenner, *Pharm. Ztg.*, 1930, **75**, 863.
[55] T. E. Wallis and E. R. Withell, *Pharm. Jour.*, 1934, **133**, 90 ; *Quart. Jour. Pharm.*, 1934, **7**, 574.
[56] Anon., *Pharm. Jour.*, 1932, **131**, 269.
[57] F. Scheermesser, *Pharm. Ztg.*, 1931, **76**, 877.
[58] I. Temesvary, *Tarsasag Ertesitöje*, 1932, **8**, 444 ; through *Chem. Zentr.*, 1933, (**1**), 1163.

[59] C. A. Rojahn and H. Herzog, *Pharm. Zentr.*, 1932, **73**, 401.
[60] J. Deiniger, *Pharm. Zeit.*, 1930, **75**, 247.
[61] C. A. Rothenheim, *Pharm. Ztg.*, 1929, **74**, 1604.
[62] W. Peyer, *ibid.*, 1932, **77**, 471.
[63] J. A. Radley, *Chem. and Druggist*, 1933, **119**, 768.
[64] G. Rattray and G. R. Milne, Lecture, Glasgow and S.W. Scotland Branch of Pharm. Soc., 1934, March 28 ; *Quartz Lamp*, 1934, **3**, 47.
[65] J. Eisenbrand and G. Siewert, *Arch. Pharm.*, 1934, **272**, 428, 441, 850.
[66] F. Wischo, *Pharm. Monatsh.*, 1930, **11**, 224.
[67] H. Neugebauer, *Apoth. Ztg.*, 1930, **45**, 80.
[68] H. Neugebauer, *Pharm. Ztg.*, 1932, **77**, 1154.
[69] H. Neugebauer, *ibid.*, 1932, **77**, 796.
[70] H. Neugebauer, *ibid.*, 1929, **74**, 626 and 1511.
[71] H. Neugebauer and A. Edwardsson, *Nordiska specialitetskomissionens ärsberättelse*, 1931, **7**, 14.
[72] C. Dhéré, Abderhalden's "Handbuch der Biologischen Arbeitsmethoden," II, 3, 4 (Berlin : Urban and Schwarzenberg, 1933).
[73] A. Marcan, "The Chemist in the Far East" (London : Institute of Chemistry, 1933).
[74] G. Klein and H. Linser, *Osterr. Botan. Zeit.*, 1930, **79**, 125.
[75] E. Beutel and A. Kutzelnigg, *Arch. Pharm.*, 1934, **272**, 849.
[76] S. G. Liversedge, *Analyst*, 1934, **59**, 815.
[77] E. Canals, S. Perrottet and P. Peyrot, *Bull. Soc. Chim.*, 1935, ii, 21.
[78] J. A. Radley, *Chemist and Druggist*, 1933, **119**, 642 ; *Manuf. Chemist.*, 1936, **7**, 257.
[79] C. Achard, A. Boutaric and J. Bouchard, *Compt. rend.*, 1933, **196**, 1757 ; *ibid.*, 1935, **201**, 629.
[80] P. W. Danckwortt, *Arch. Pharm.*, 1936, **274**, 184.
[81] J. Khouri, *Ann. Falsif.*, 1935, **28**, 582.
[82] E. J. Kocsis and F. Nemeth, *Zeit. anal. Chem.*, 1937, **111**, 188.
[83] C. C. Fulton, *Jour. Amer. Pharm. Assoc.*, 1937, **26**, 726.
[84] E. Canals, S. Perrottet and P. Peyrot, *Bull. Soc. Chem.*, 1935, **2**, 21.
[85] A. Boutaric and J. Bouchard, *Compt. rend.*, 1936, **203**, 167.
[86] M. Prost, *ibid.*, 1936, **202**, 1047.
[87] M. Haitinger, *Microchem. Acta*, 1937, **1**, 1.
[88] P. Antonio, *Boll. Chim. farm.*, 1938, **77**, 209.
[89] M. Déribéré, *Ann. chim. anal. chim. applic.*, 1936, **18**, 119.
[90] M. S. Dunn and W. H. Kimmer, *Amer. Jour. Pharm.*, 1937, **109**, 498.
[91] S. K. Crews, *Quart. Jour. Pharm.*, 1936, **9**, 434.
[92] S. K. Crews, *ibid.*, 1937, **10**, 369.
[93] Anon., *Ind. Chem.*, 1936, **12**, 461.
[94] L. Zechner and R. Gager, *Pharm. Monatsh.*, 1937, 188.
[95] E. J. Kocsis, *Magyar gyóg. Társ. Ertes.*, 1935, **11**, 99.
[96] C. A. Rojahn, M. Singer, W. Schill and A. Hellrung, *Pharm. Zentr.*, 1935, **76**, 453.
[97] B. Reichert and H. Böhme, *Deut. Apoth.-Ztg.*, 1937, **52**, 1106.
[98] E. Merck, Anm. M., 129,855.
[99] E. Merck, Anm. M., 133,667, IV a/30i(10).
[100] Mannich, *Deut. Apoth. Ztg.*, 1926, **41**, 694.
[101] S. Isermann, E. Ohlsson and J. W. Orelup, U.S.P. 2,102,712.
[102] H. Neugebauer, *Apoth. Ztg.*, 1935, **50**, 1112.
[103] K. Szahlender, *Arch. Pharm.*, 1935, **273**, 234.
[104] A. Kuhn and G. Schäfer, *Apoth. Ztg.*, 1935, **50**, 1800.

[105] A. Kuhn and G. Schäfer, *Pharm. Ztg.*, 1935, **80**, 1253.
[106] J. Khouri, *Ann. Méd. Légale*, 1936, **16**, 249.
[107] A. Boutaric and J. Bouchard, *Ann. Soc. Sci. Brussels*, 1936, **56B**, 35.
[108] W. D. Raymond, *Analyst*, 1938, **63**, 478.
[109] N. L. Allport and S. K. Crews, *Pharm. J.*, 1935, **135**, 8.
[110] F. Hoeke, *Chem. Weekblad*, 1938, **35**, 316.
[111] H. J. Vlezenbeek, *Pharm. Weekblad*, 1937, **74**, 127.
[112] M. Pesez, *J. Pharm. chim.*, 1938, **27**, 247.
[113] M. Bachstez and J. Cavallini, *Chim. e Ind.*, 1937, **19**, 126.
[114] F. J. Kaiser, *Rec. Trav. chim. Pays-Bas*, 1938, **57**, 117.
[115] E. H. Maechlin, *Ind. Eng. Chem. Anal.*, 1938, **30**, 586.
[116] B. K. Johnson, *Jour. Sci. Inst.*, 1934, **11**, 384.
[117] C. A. Rojahn, *Pharm. Zentr.*, 1937, **78**, 81, 127, 146 ; C. A. Rojahn and D. Mehta, " Indentifizierung von 337 Drogen mit Hilfe chemischer Gruppenreaktionen und der Kapillar- und Luminescenz Analyse " (Halle), 1937.
[118] Anon., *Chem. Ztg.*, 1939, **63**, 99.
[119] P. v. Aufschnaiter, *Pharm. Monat*. 1938, **19**, 99.

ADDITIONAL REFERENCES.

R. Freudweiler, *Pharm. Acta Helv.*, 1933, **8**, 147, 155, 190 (General).

T. Yokio and M. Ishii, *Jour. Pharm. Soc. Japan*, 1929, **49**, 475 (Detection of α- and β-naphthols).

E. A. Lum, *Pharm. Jour.*, 1932, Dec. 31 (Confirms previous findings for rhubarbs).

B. von Issekulz, *Pharm. Monatsh.*, 1933, **14**, 30 (Rhubarbs).

Gehrmann, *Apoth. Ztg.*, 1933, **48**, No. 80 ; *Deut. Apoth.*, 1933, **2**, 176 (*Oleum hyperici*).

F. Sonntag, *Apoth. Ztg.*, 1935, **50**, 339 (Examination of *Oleum hyperici*).

P. Kämpf, *Pharm. Acta Helv.*, 1933, **8**, 170, 214 ; P. Kämpf, *Diss. Bern*, 1932 (Detection of adulteration of drugs by capillary analysis).

G. Fodor and A. Kichler, *Pharm. Monatsh.*, 1930, **11**, 29 (Galenicals).

K. Leupin, *Pharm. Acta Helv.*, 1933, **8**, 166 (Detection of adulteration of *Crocus* using the fluorescence microscope).

A. Mingoia, *Ann. Chim. applic.*, 1933, **23**, 318 (Action of ultra-violet light on solutions of alkaloids).

T. J. Rumi, *Rev. Centro Estud. Farmac. Bioq.*, 1933, **23**, 98 (General).

L. Rosenthaler, *Pharm. Ztg.*, 1933, **78**, 1063 (Galenicals).

S. Malowan, *Chem. Ztg.*, 1933, **57**, 76 (General).

L. van Itallie, *Pharm. Weekblad*, 1929, **66**, 872 (General).

T. Wikander, *Pharm. Ztg.*, 1930, **76**, 1360 (General).

G. Heinrici, Diss., Halle a. S., 1932 (General).

M. Haitinger, *Mikrochemie*, 1935, **16**, 353 (Detection of alkaloids by fluorescence microscopy).

G. R. Levi and F. Castelli, *Gazz.*, 1938, **68**, 459 (Fluorescence chromatography of opium alkaloids).

H. Eschenbrenner, *Apoth. Ztg.*, 1930, **45**, 1081 (Detection of arbutins).

W. E. Naylor and A. Surfleet, *Pharm. J.*, 1936, **136**, 261 (Distinction between B.P. precipitated chalk and prepared chalk in McLean's powder).

V. Zanotti, *G. Farm. Chim.*, 1936, **85**, 40 (Capillary analysis of tinctures).

G. E. Trease, "Textbook of Pharmacognosy," 1938 (Miscellaneous applications).

CHAPTER VI.

FOODS AND FOOD PRODUCTS.

Oils and Fats.

THERE is a tendency on the part of some workers to make the fluorescence test an absolute criterion of purity of certain oils. Although it gives very valuable evidence, the results of the test must be taken in conjunction with other physical and chemical tests before a reliable decision can be made whether the samples are genuine or not. This point, which cannot be emphasised too strongly, applies to practically every branch of fluorescence analysis (see especially the work of P. Barbade [131] and A. Bömer [142]). On the other hand, many instances will be found [185, 196] where qualitative tests are sensitised considerably if carried out in ultra-violet light (see especially Sugars).

Olive Oil.—The fluorescence of olive oil from various sources has been examined by many investigators, some of whom record the visible appearance in filtered ultra-violet light, whilst others have examined the emission and absorption spectra. A large proportion of the work has been directed to the detection of refined oil in virgin oil, and according to R. Marcille,[1] A. Baud and Courtois,[2, 3] S. Musher and C. E. Willoughby,[4] and to Frehse [5] and others,[193] this detection is possible, and is especially facilitated by use of a spectroscope or spectrophotometer.

With *virgin olive oil* a yellow fluorescence is obtained, but exposure to sunlight or to the rays of the lamp for any considerable time, causes this to disappear. Oxidation induced by long exposure to the air, or by fairly strong heating, causes a change in the fluorescence from a yellow to a bluish-green or violet tone, and this is probably a contributory cause of the difference between the colour of the fluorescence of refined olive oil and of virgin olive oil; according, however, to J. Becker [194]

photochemical action plays a more important part in the case of vegetable oils. The violet fluorescence is particularly noticeable in oils which have been refined under pressure or obtained by solvent extraction. Treatment with hydrogen peroxide, ozone or bromine, produces no alteration in the colour of the fluorescence of the oil, but after treatment with benzoyl peroxide a light whitish-blue tone may develop.

Frehse [5] has examined a number of olive oils in filtered ultra-violet light of wave-lengths between 3650 and 4040 Å. and of 3240 Å. He found that *refined sulphur oils* (oil extracted from the pulp with carbon disulphide) showed a more pronounced and greenish fluorescence than the refined olive oils. Crude oils from Spain, Algeria, St. Dennis du Sig, Tunis and Italy gave an orange fluorescence. The addition of 5 per cent. of pulp oil to the other oils replaced the orange by a green colour, a similar result being also obtained by the addition of 10 per cent. of refined oil. The green fluorescence was also developed on the addition of native (French) oil, but only when this addition was made in quantities exceeding 15 per cent.

G. Lunde and F. Stiebel [139, 140] have examined 300 samples of olive oil, using a Pulfrich step-photometer and red, green and blue filters. The thickness of the oil layer is plotted against the intensity of the fluorescence, and they find that all virgin olive oils give either a red or a yellow fluorescence, a band at 6990 Å. being observed. If the oil has been refined or extracted this band is absent, and the blue component is then considered as the best measure of the amount of refined oil present. These workers consider that the direct observation of the fluorescence without special apparatus allows the detection of refined oil only when it is present to the extent of over 35 per cent. With their apparatus, however, the detection of the presence of 10 per cent. of refined oil is possible.

It is not unknown for substances which give a dark purple or brown masking fluorescence to be added to adulterated virgin oils (*e.g.*, containing tea-seed, arachis or sesame oils or refined olive oils) ; these give similar analytical figures to genuine virgin olive oil, except for the iodine value of the unsaponifiable matter, which is lowered. T. T. Cocking and S. K. Crews [141] find, however, that this fluorescence may be removed by treating 100 c.c. of oil with five 1 grm.-portions of decolorising charcoal and

examining the oil after filtration. Genuine oils appear very faint blue instead of golden-yellow in ultra-violet light, and oils containing over 5 per cent. of adulterating oil have an unmistakable blue fluorescence, even if the masking fluorescence was present originally. These workers confirm the general utility of the test, and they show that the blue fluorescence produced by heating oils at 300° C. survives the carbon treatment. W. Ciusa [214] confirms these results and finds that expressed oils prepared from damaged olives may show a blue-violet fluorescence, but that this is removed by treatment with carboraffin, the oil then appearing the same as a normal expressed oil.

G. Lunde and his co-workers [144] have also followed the extinction-coefficients of refined and virgin olive oils, the value for the former being 35 to 55 at 2750 Å., compared with 10 to 22 for the virgin oils, and 54 to 90 for refined and extracted oils. The fluorescence follows the same trend, and both are increased by treatment with heat or by neutralisation with warm 8 per cent. sodium hydroxide solution, but are decreased, however, when the neutralisation is carried out in an acetone-ethyl alcohol mixture using a 0·4 per cent. solution.

R. Stratta and A. Mangini [6] have examined a number of Italian olive oils in layers over 3 mm. in thickness. They found that virgin Apulian oils acquire a lemon-yellow to orange fluorescence changing, after an exposure of 30 minutes, to a natural sienna colour. Sansa oils, solvent-extracted and refined, give a sky-blue fluorescence, unchanged on exposure, whilst refined "lampante," *i.e.* washed and filtered oil, assumes a dull greenish-blue fluorescence. They claim that 1 per cent. or more of refined oil in virgin olive oil changes the colour of the fluorescence, which becomes almost white with 10 per cent., pale blue with 30 per cent., and a brilliant sky-blue with 60 per cent. or more of the refined oil. It is, however, very unlikely that additions so small as 1 per cent. could be detected in this way with ordinary simple apparatus, and attention should also be drawn to variations in the colour of refined oil as specified by different workers as a further example of the uncertainty attached to the method when used for small amounts.

S. Musher and C. E. Willoughby [4] have noted that *second-pressing oils* are darker in fluorescence than those of the first pressing. They used a spectrophotometer in conjunction with

the lamp, and state that 5 per cent. of refined olive oil in the virgin oil can be detected in this manner although they consider that 65 per cent. is the minimum amount detectable by direct observation. Virgin olive oil heated at 300° C. for 30 minutes develops a fluorescence which then resembles that of a virgin oil containing from 5 to 10 per cent. of refined oil. This method should be useful for the comparison of a sample submitted with the bulk of oil delivered, but obviously, should be used with caution unless it is known for certain whether or not the oil has been heated. An observation of M. Gisondi [132] that the ripeness of the fruit before pressing and the age of the oil cause some variation in the fluorescence colour, again emphasises the need for caution in this branch of the work.

Origin of the Fluorescence.—The yellow-coloured fluorescence of virgin olive oils is ascribed by A. le R. Glantz [7] to carotene which is destroyed during the refining processes. He tested olive oils from California and concluded that this destruction occurs during the combined heating and pressing processes. The fluorescence-spectrum of virgin olive oils shows a characteristic red band at 6690 Å. which is absent from refined oils, but which reappears on the addition of chlorophyll. It is stated that the presence of so little as 10 per cent. of virgin olive oil in refined oil can be detected by the observation of this band. The blue fluorescence of the oil appears to be independent of its chlorophyll content, and the addition of annatto or of carotene brings back the yellow fluorescence of virgin olive oil once more. In this connection it should be mentioned that D. Cortese [195] was able to simulate the fluorescence of a pressed oil by adding carotene or chlorophyll to a refined oil. J. Guillot [209] examined the spectra of the fluorescence of the various oils, and was able to show that virgin and refined oils both contain a component having a blue fluorescence, but that natural or added pigments (chlorophyll, xanthophyll and carotene) have a yellow fluorescence which may mask the blue colour.

Musher and Willoughby,[4] on the other hand, consider that the variation in the colour of the fluorescence of the oil is due entirely to chlorophyll, and cite the fact that the first pressings of Californian oils resemble the second pressings of oils from Europe, more oil being extracted in the first case and, correspondingly, more chlorophyll. The detection of adulteration

is therefore difficult if annatto and carotene are used, for the chemist can readily detect annatto but not carotene. From this it follows once again that the results obtained under the lamp must be interpreted with care, and this opinion is endorsed by E. R. Bolton,[8] who has also pointed out that certain oils (*e.g.*, tea-seed oil) used for adulteration, have a fluorescence similar to that of olive oil; this particular difficulty has, however, since been eliminated by Cocking and Crews (p. 142). A. G. Nasini and P. de Cori,[9] also, have worked on the addition of chlorophyll and carotinoids, and have compared the absorption spectra and the fluorescence of solutions of chlorophyll in refined olive oils and in other natural oils. They consider that addition of chlorophyll can cause refined olive oil to assume the fluorescence shown by virgin olive oil, a statement with which Musher (*supra*) disagrees.

Van Raalte[10] suggests that the fluorescent phenomena are related to the presence of vitamins, a suggestion supported by current theories of carotene as the precursor of a vitamin, and also by the work of J. A. Pierce,[11, 12] and by G. Loew,[13, 14] who states that vitamin C, or a related substance, is the seat of the luminescence (*cf.* p. 149). Pierce found that the blue fluorescence of olive oils filtered through Fuller's earth is not changed back to yellow by the addition of carotene or chlorophyll, although exposure to ultra-violet light has this effect.

Spectrographic analyses of the fluorescence of various vegetable oils have been carried out by H. Marcelet and H. Debono,[15, 16] and by J. Guillot.[210] Olive, soya bean, sesame, maize, grape seed, arachis, tea, cotton-seed, and argan oils produce different emission and absorption spectra in the ultra-violet region. They consider that 5 per cent. is the limit for the detection of the refined oil in virgin olive oil, but 10 per cent. of soya bean, maize, or grape-seed oil gives inconclusive results. Tunisian, Algerian and Moroccan olive oils give a mauve, yellow, apricot, green, whitish or chocolate coloured fluorescence, and this fluorescence bears no relation to the natural colour of the oil. Since in all cases the spectrum of the fluorescence showed a luminous field between 5200 and 6900 Å., the maximum intensity being between 6650 and 6900 Å. for most of the oils, this range may be regarded as characteristic for olive oils; these rays were, however, only obtained with oils showing a mauve, apricot or brick-red fluorescence.

E. Lewkowitsch,[17] who examined a number of fatty oils, including olive oils, in order to ascertain whether they possess any characteristic ultra-violet absorption spectrum, concluded that this is the case, but that so many factors enter into the observations that the identification of absorption bands is difficult. Guillot, however, was able to obtain useful indications of the origin and methods of processing of olive and other oils from the absorption-coefficient at the wave-length 2700 Å.

To sum up, although the lamp does not give results which lead to the absolute identification of adulteration, it can give valuable assistance by indicating the origin and history of the sample,[18] especially on comparison with specimens of known origin and purity. Thus Croner [19] has tabulated the fluorescence colours of various oils and fats both before and after heating to a specific temperature, and he considers that a blue luminescence at the surface of a fatty oil indicates that it has been heated above 150° C. or that it contains, or is contaminated with, mineral oil.

Other Edible Oils.—The oils now obtained commercially by solvent-extraction include linseed, cotton-seed, sunflower-seed, soya bean, palm kernel, copra, rape-seed, maize, groundnut and castor-seed oils. A solvent-extracted oil, or an oil that has been refined under pressure, generally has a violet fluorescence. Oils obtained by expression usually show a yellow or green fluorescence. Walnut, sesame, almond and sunflower seed oils appear yellowish, the last being paler and duller than the others. Hazelnut oil appears very weak blue, and castor oil has a clearer blue luminescence. Maize oil fluoresces yellowish-green, pine oil very slightly green, and soya bean oil a dull dark green (see also Forjaz [20]). L. Francesconi and L. Pinoncelli [145] have examined the fluorescence and the physical and chemical properties of vegetable oils on exposure to ultra-violet light in an atmosphere of nitrogen or carbon dioxide. They state that the fluorescence turns blue and that the oils undergo oxidation. Castor, coffee and arachis oils have been examined by A. P. Forjaz.[146]

H. Marcelet [21] examined samples of *cod-liver oil*, representative of those used in pharmacy and industry, by placing a drop of the oil on a vertical sheet of non-fluorescent paper or glass, the streak from the falling drop being examined in ultra-violet light with a sheet covered with non-fluorescent animal

charcoal as background. The colours of the drops ranged from pale or golden-yellow to chestnut-brown, according to the quality of the oil, and this fluorescence does not disappear on heating, although it is destroyed by hydrogenation. The yellow fluorescence may possibly be associated with, or due to, the presence of vitamin A, which is present in the oil in relatively large quantities (*vide* p. 149).

C. A. Morehouse,[22] who examined the fluorescence spectra of a number of cod-liver oils, observed a bright band extending from 5460 to 4360 Å. This was present also in solutions of cod-liver oil in alcohol, and in alcoholic solutions of cholesterol. M. Auerbach[133] has found the Callophane (p. 22) a suitable instrument for the detection of shark oil in cod-liver oil, and for mineral oils in neatsfoot oil. R. H. Common[216] finds that sperm oil has a greenish-blue fluorescence, but that this is masked by the bright grass-green fluorescence of cod liver oil or shark oil, unless these are present to the extent of less than 50 per cent.

A. Wagner[143] has examined a number of oils (see p. 329, Paints) and finds that solvent-extracted soya bean oil can be differentiated from the pressed oil and that the residual cakes are also readily differentiated, those extracted with benzene and a chlorinated solvent having a green or blue fluorescence, respectively. One of us (J. A. R.) has examined arachis oils intended for use in lotions, and the presence of a mineral oil in one adulterated sample was very readily detected by its brilliant blue fluorescence (see also Guillot[210]). R. G. Harry[215] finds that date stone oil has a bluish-purple fluorescence, whereas the fatty acids separated from the oil have a greenish-blue fluorescence.

Fatty acids examined by B. Lustig and G. Botstiber[187] and A. Niethammer[188] had colours as follows: Acetic and propionic, colourless; butyric, weak yellow; palmitic, pale yellow-grey; stearic, white with a violet tinge; oleic (pure), strong violet; capronic, red-violet; erucic and lauric, vivid violet; formic and lactic, pale lilac.

Reactions for *cholesterol and its related compounds* which are rendered more sensitive if carried out in ultra-violet light are:[185] (1) A mixture of equal parts of concentrated sulphuric acid and a solution of cholesterol in chloroform is shaken, when a red colour having a green fluorescence results (Salkouski). (2) Cholesterol heated with a 90 per cent. solution of trichloracetic acid

develops a green fluorescence changing to blue in 24 hours (Hirschsohn). (3) A red colour and green fluorescence is obtained if a solution of cholesterol in glacial acetic acid is warmed for 5 minutes with acetyl chloride and zinc chloride (Tschuggern); sensitiveness 1 : 80,000. (4) See Rosenheim's reaction below.

Isocholesterol (the Schulze-Liebermann-Burchard reaction [189]). A solution in warm acetic anhydride is cooled and a drop of concentrated sulphuric is added, when a yellow colour turning red and then showing a green fluorescence results.

Oxycholesterol (Rosenheim [190]). The sample is warmed with dimethyl sulphate, when cholesterol and oxycholesterol give a red and purple colour, respectively; in chloroform or benzene these appear blue and (on addition of ferric chloride) green, in the respective cases.

Irradiated Ergosterol (Meesemaecker [191]). (1) A fresh solution in chloroform produces a transient yellow colour with anhydrous phosphorus pentoxide and, after a few days, a green colour having a green fluorescence. (2) In J. Bruckner's test for ergosterol [168] the sample is dissolved in 2 c.c. of benzene, and 1 c.c. of acetic anhydride is added, together with 0·5 c.c. of acetone, 0·5 grm. of anhydrous zinc chloride and a crystal of copper acetate. A blue-violet colour and a red fluorescence is obtained in the presence of 0·001 mg. per c.c.

Isoergosterol (Rosenheim and Callow [192]). A solution of ergosterol in chloroform produces a green colour and fluorescence when irradiated in the presence of a solution of 25 grms. of mercuric acetate in 100 c.c. of nitric acid (sp. gr. 1·42).

Butter, Milk and Other Farm Products.

Butter and Margarine both fluoresce under the lamp, butter with a yellowish colour, and margarine with a strong blue. Butter and horse-fat treated with sulphuric acid show a very strong fluorescence, whilst mutton fat luminesces strongly after treatment with Fuller's earth; according to J. F. Carrière,[23] this may be due to a small amount of bituminous material present in the Fuller's earth.

H. P. Stadler [24] and also M. Haitinger and his co-workers [25] have tabulated the fluorescence colours of butters and various

artificial edible fats, and their mixtures. They recommend the use of solutions of the fat in petroleum spirit, a solution with a feeble yellow fluorescence being obtained with butter, whilst with margarine the fluorescence is a strong bluish colour. So strong is the blue fluorescence of the margarine that the presence of 15 per cent. can easily be detected in butter, especially when a solution of a pure butter is used as a standard for comparison. A. de Clercq [217] has attempted, without success, to detect the adulteration of butter with less than 10 per cent. of a vegetable or animal fat by measuring the intensity of the reflected ultra-violet light.

Vitamins and Fluorescence.—R. S. Morgan and K. MacLennan [26] have devised a method (p. 64) whereby the actual brightness and colour of the fluorescence is measured in terms of the three primaries, red, green and blue. They examined a number of fats containing vitamin A and found that they all gave a yellow fluorescence, which seemed to be closely connected with the presence of the vitamin (*vide supra*). P. R. Peacock,[27] however, noted a number of discrepancies between the vitamin A content and the fluorescence, and this was confirmed by Morgan and others, who suggest that another substance having a similar fluorescence is formed in the fat. There appears to be no connection between vitamin D and the fluorescence, and the difference between the fluorescence colours of margarine and butter does not seem to be explainable by the difference in vitamin A content alone. When, however, margarine is coloured by the addition of red palm oil to give it the appearance of butter, and when sufficient unsaponifiable matter from cod-liver oil is added to bring the vitamin A potency up to that of butter-fat, it is indistinguishable from butter in daylight, or in ultra-violet light.

The blue fluorescence of margarine is depressed by the addition of certain pigments, and addition of the unsaponifiable matter from cod-liver oil markedly increases the brightness and, at the same time, decreases the blue colour of the fluorescence. Addition of certain vegetable oils which fluoresce with a bright blue colour, or of oleo and jus, which have a greenish fluorescence, may also modify the fluorescence of margarine in a similar way. It is interesting to note that J. W. Woodrow and A. R. Schmidt [169] have attempted to correlate the fluorescence of butters, spinach, tomatoes and other foodstuffs with the vitamin A or carotene

content ; they find the lines 5300-7300 and 4100-5100 Å. to be common to the fluorescence of both classes of substance.

F. H. Cohen [202] and B. Josephs [203] discuss the relationship of fluorescence to the determination of vitamin B_2, and R. A. Peters [204] has based a discussion of the constitution of vitamin B_1 on the sky-blue fluorescence produced on oxidation (*cf.* p. 68). H. W. Kennersley and co-workers [218] find that the blue fluorescent compounds are biologically active, and suggest the name quinochrome for them.

E. Nováček and J. Hökl [28, 29] consider that it is possible to detect additions of small quantities of *foreign fats in butter.* They examined mixtures of artificial fats and beef tallow with 5 to 90 per cent. of butter, the mixtures being poured into petri dishes of non-fluorescent glass and examined under the lamp. The quantities used were controlled by the butyro-refractometer and the Litterscheid polarisation microscope. In all cases of mixtures with fresh butter and artificial fats it was possible to detect 5 per cent. of the latter under the lamp, by the use of pure butter for comparison. It was also found that the age of a sample and its degree of rancidity could be gauged to some degree from the extent of the change in the colour of the fluorescence from yellow to blue ; with artificial fats a dirty colour develops on ageing without any loss of fluorescence. Pure beef tallow has a dirty white fluorescence with a yellowish tinge, and on ageing this changes to light grey or weak violet.

O. Laxa [30] has examined solid samples of butter, margarine, and mixtures of the two in order to determine the least amount of margarine in admixture with butter which may be detected under the lamp. He considers that in the solid state, 20 per cent. of margarine in butter is recognisable, but that for a mixture dissolved in ethyl ether, amyl alcohol, benzene or acetone, 33 per cent. of margarine is the approximate limit ; a 50 per cent. mixture has a definite bluish shimmer at the edges. Melted butter and margarine shaken with glycerol show a bright canary-yellow, and a bright dirty brown fluorescence, respectively, the latter being easily visible in mixtures of butter and margarine containing 25 per cent. of the latter. A. de Clercq [217] failed to detect adulteration of butter with less than 10 per cent. of an animal or vegetable fat.

J. Lenfeld and co-workers [31-35] examined specimens which

had been kept at a low temperature for some months, and confirmed the fact that age, rancidity, pretreatment and admixture with foreign fats all produce changes in the fluorescence colour; he therefore concludes that there is no advantage to be obtained by the use of a solution of the fat. One of the most important points of his work is, that he often found that the fluorescence colours shown by various samples examined under the Müller and Hanovia lamps to be different, and this fact emphasises the need to work with specified ranges of the ultra-violet spectrum if different workers are to obtain comparable results. The portion used for examination should be taken from the middle of the sample, as the action of light and air often modifies the fluorescence-colour at the edges, and according to Haitinger and to O. Gerngross and M. Schulz,[36] butter may develop a dirty greyish-yellow fluorescence, with a faint bluish shimmer at the edges which is even more pronounced in any cracks.

Another difficulty in the use of the method for the detection of artificial fats, such as margarine in butter, has been indicated by J. Hökl,[37] who found that tallowy butter develops a fluorescence similar to that produced by addition of the former fats to fresh butter. Rancid butter, however, often retains unmodified the normal fluorescence of fresh butter.

Mention should also be made of the work of G. W. Baker and S. Taubes,[55, 129] since this is a valuable summary of the present position regarding milk and butter. They conclude that the yellow fluorescence of fresh milk from the cow, goat or sheep cannot be attributed to the fat. In skim or whole fresh milk the fluorescence changes gradually to blue on standing at ordinary temperatures (unless the milk has previously been boiled or preserved with formalin), but the yellow colour may be regenerated temporarily by shaking, although the blue returns on standing, especially if air is removed from the tube. Control experiments in inert gases indicated that oxygen is the active agent responsible for the regeneration of the yellow colour, and the mechanism of the change appears to be analogous with the reductase test. This work is discussed further by Radley on p. 159.

Coconut-fat has an intense blue fluorescence, and more than 5 per cent. is detectable in butter-fat, or in the absence of a pure specimen for comparison, more than 15 per cent.

So far as other animal fats (from the cow, sheep and goat) are concerned, when freshly rendered these differ only slightly in fluorescence from butter-fat, but after about a month they develop a definite dirty blue fluorescence. Artificial colouring matters, which may mask the fluorescence, are removed by treatment with boiling water or with 3 per cent. of animal charcoal.

It is a tribute to the method that out of 870 genuine samples, mostly native " samneh " (rendered butter-fat), 99 per cent. showed the characteristic creamy-white to yellow colour, whilst all but 3 of the 89 adulterated samples were distinguishable by the blue appearance. In Baker's opinion the test is " of considerable practical value ; more so, perhaps, than any other single routine test, with the exception of the determination of the volatile fatty acids." Incidentally, pure Syrian samneh may be distinguished from " mixed " samneh, which is dutiable in Palestine.

Lard.—E. Nováček and J. Hökl [29] examined lards and found that Czecho-Slovakian lard appears more yellow in ultra-violet light than in daylight ; since American lard is less yellow and more violet, 40 per cent. of Czecho-Slovakian lard is detectable in it. These workers consider also that 20 per cent. of Dutch lard is detectable, or if a standard lard is used for comparison, then 5 to 10 per cent. is detectable ; 40 per cent. of beef tallow can also be seen in admixture with lard, whilst 40 to 70 per cent. of " Sana " margarine imparts a dirty white fluorescence to American lard. Admixture of 20 per cent. of horse fat is visible in American lard, and in some cases where mixtures of vegetable fats and horse fat produce no change in the refractive index, the ultra-violet light test will distinguish additions of so little as 10 per cent.

A. van Raalte,[38] on the other hand, has stated that no fluorescence is to be observed in most ordinary lards, but that with refined pig's fat a blue or violet luminescence is obtained. This was also contradictory to the results of A. van Druten,[39] but later, the two investigators co-operated [40] and showed that the cause of the discrepancy was the different conditions under which they carried out the earlier experiments. This again emphasises the importance of standardised conditions.

It may be safely concluded, therefore, that the behaviour of lard in ultra-violet light gives valuable indications as to

its purity, and to the previous treatment to which it has been subjected. F. Weiss [41] attempted a rough classification of lards in this way by division into six classes, depending to some extent on the type of lard, and on the manner of purification :—

1. Samples prepared in the laboratory, and also certain good commercial samples showing a faint yellow, white or blue fluorescence, or else none at all.

2. The same samples after exposure to light and air, the fluorescence being modified, *viz.*, a blue colour appears in the upper layer.

3. The same samples which, after treatment with activated charcoal, are not changed by this process.

4. Samples treated with superheated steam at about 150° C.; these have a characteristic clear blue fluorescence.

5. Lards refined by the Dutch method, which also show a characteristic blue luminescence.

6. Lards containing " unrefined white grease " ; these have a white, blue or violet ringed fluorescence, which decreases in intensity in the lower layers.

These results are, in general, in agreement with those of Braunsdorf,[42] who wisely confined his examination to lards prepared by himself, and therefore, of known history. It is important to note that a yellow or orange tinge in the fluorescence may be due to a similar colour present in the lard when viewed in daylight, and that this may be removed by heating above 105° C. R. Nesini [147] has also examined native lards, and considers that the fluorescence depends essentially on the temperature at which it is produced. Native fats heated at 150° C. or over cannot be distinguished from American fats under the lamp, and hence it is not possible to differentiate a pure native lard from a mixture in this way.

Van Raalte,[43] who treated lards with alkali carbonates, alkaline, earth hydroxides, Fuller's earth or steam, observed that lards which had previously shown no special fluorescence developed a white, green or blue colour. This led to the suggestion that the fluorescence produced in fats and oils by refining is due to the removal or destruction of some constituent which inhibits fluorescence, and a vitamin or a sterol has been suggested (*cf.* p. 148). This " de-activating " body is destroyed by heat, but

not by freezing, or by treatment with benzoyl peroxide or active carbon.

The addition of 10 per cent. of cotton-seed oil imparts a bluish shimmer to the fluorescence, and in thin layers, a solution of the mixture in petroleum spirit has a blue colour. A similar blue colour is also obtained after the addition of sesame or arachis oils, but, according to Haitinger and his co-workers,[25] the admixture of beef fat with lard cannot be detected by this method. E. Feder and L. Rath, [44] and also Bengen and Gröning,[45] mention the intense bluish-violet fluorescence shown when white grease is present, and Bengen considers that the presence of 0·001 per cent. of "white grease" may be detected accurately. Finally, J. Grossfeld [46] finds that there is no apparent relation between the fluorescence of lard and its caprylic acid content.

It will be seen from the above facts that, as in the cases of butter and olive oil, it is not yet possible to arrive at a final opinion as to the cause of the fluorescence in lard, and that the method if used with discrimination and with known standards, is a valuable aid to analysis.

Cocoa Butter.—Among the other foodstuffs examined in ultra-violet light is cocoa butter, and G. Popp [47] mentions that it fluoresces with a yellow colour, which is almost eliminated by addition of coconut oil. Six hours of exposure under the lamp also completely removes this colour, although it has no effect on the green colour of Borneo tallow; this difference forms the basis of a method for the detection of the addition of Borneo tallow to cocoa butter, and has been elaborated by A. Knapp and his co-workers.[48] J. Ripert [49] recommends extraction of the fat with chloroform, removal of the solvent and then decolorisation of the butter with 100-volume hydrogen peroxide at 90° C. After this treatment the butter fluoresces with a bluish-violet colour, but additions of Rotterdam butter or of "beurre Radisson" produce green and pink tints, respectively.

W. Schmandt [50] (after Haitinger, Jörg and Reich [174]) examined a 2·5 per cent. solution of the cocoa butter in non-fluorescent petroleum spirit. The solution appears dark or greyish in colour if mechanically-expressed butter only is present, but 1 per cent. of cocoa extract or waste butter causes the development of a bright blue fluorescence.

W. T. Field [51] confirms the majority of these observations,

but prefers a 0·1 per cent. solution of the butter. He considers, however, that the detection with certainty of less than 25 per cent. of extracted butter admixed with pressed butter is not possible. As the fluorescence of different butters varies in quality, as well as in intensity, the solution of the butter cannot be diluted to match a standard fluorescence and this dilution then taken as a measure of the intensity of the fluorescence. Raw butter heated to 150° C. for 1 hour gave a fluorescence similar to that given by a butter from roasted beans. A. Castiglioni [201] adds 3 c.c. of glacial acetic acid to 0·5 grm. of the melted butter; with solvent-extracted butters the acid layer has a yellow-green fluorescence, but with pressure-extracted products it has none.

The absorption and fluorescence spectra of various vegetable fats have been examined by W. Sproesser,[52] who finds that cocoa butter dissolved in chloroform has a weak absorption maximum between 2700 and 2800 Å. H. P. Kaufmann,[53] who has also worked on similar lines, considers that the absorption spectrum of cocoa butter is of little use for the identification of foreign fats, but that it may be of service when pure substances are used. The fluorescent properties of cocoa butter have been ascribed by D. van Roon [54] to one or more glycerides present in the pure fat, but small traces of substances (*e.g.*, metal soaps) introduced on refining may also contribute to the colour observed.[134]

To sum up, if the cocoa butter is non-fluorescent, then it is certainly not solvent-extracted, or else it has been obtained from very lightly roasted beans. A cocoa butter with only a slight fluorescence allows no conclusions to be drawn with certainty, and a strong fluorescence shows that the butter has been solvent-extracted, or else that it is contaminated with mineral oil from some other source.

Milk.—The investigation of milk and dairy products under the lamp yields a number of interesting facts. Both G. Popp [47] and J. Volmar [67] state, for example, that the intensity of the yellow fluorescence observed in milk is directly proportional to its fat content, although this statement has been contradicted by K. Schützler,[56] Kieferle [57] and others (*vide infra*).

According to O. Gerngross and M. Schulz [58] milks from the cow, sheep and goat and, to a less extent, human milk, bitch milk, and milk from the lioness, mare and ass all show a yellow fluorescence. The light yellow fluorescence of mare's

and asses' milk turns blue after a very short time. In this connection Baker [59] has also noted that with cow's milk the blue colour starts from the bottom and spreads upwards, turns yellow again on shaking, and returns to blue once more on standing. The colour of cow's milk is not always the same, and intermediate shades are obtained, ranging from intense canary-yellow, with a slight fluorescence, to greenish and ivory tones. Not only is it clear that the intensity of the fluorescence has no connection with the content of fat, but also, there appears to be no relation between it and the total solids. J. Spolverini,[60, 61] who worked on milk from the cow, goat and ass, noted that each has a typical fluorescence which serves as the basis of a method of quantitative and qualitative examination. F. Tallo and B. Albanese [62] also consider that it is possible to distinguish milks from different animals surely and rapidly, but samples of cow's milk examined by one of us (J. A. R.) from a first-class herd giving Grade A, T.T. milk, gave colours which correspond with the colour chart obtained by these workers for other milks.

In dairy treatment the substance responsible for the fluorescence goes into the skim, or butter-milk, and whey. Only small amounts are found in the cream or butter, and freshly-separated proteins, washed with water, do not contain it. Whatever the fluorescent material may be, it is only slightly lipoid-soluble, and it is resistant to oxidation or to boiling in acid or neutral solution, although it is destroyed in alkaline solutions of *p*H 9·0. On acidification it is regenerated, the maximum intensity being observed at about *p*H 6·0 and another minimum at *p*H 3·0. Subsequent neutralisation causes the fluorescence to return again.

Exposure directly to sunlight changes the colour of the fluorescence to white and then to bright blue. A similar effect is observed with diffused daylight, but the change takes 24 to 48 hours for completion, whilst in the dark, the yellow fluorescence of milk preserved with formaldehyde persists for months; Mezger [66] confirmed that this colour change is reversible.

The Origin of Fluorescence in Milk.—The yellow-coloured fluorescence was first ascribed to urobilin, although the blue fluorescence of irradiated whey is not identical with that of urine (see p. 230). It is known that urobilin is present in the milk of animals in their first lying-in, and that its content

gradually decreases, until finally the milk of frequently-milked animals contains no trace. There is no difference between the fluorescence of milk from fresh-milking cows and old-milking cows. Danckwortt states that he has investigated this decrease in urobilin using the quartz lamp, but so far without result. Kieferle [57] mentions that evening milk from a cow is appreciably less fluorescent than morning milk from the same cow. C. Schuetzler [63] considers that the colour of milk is due to vegetable pigments and albuminoid compounds, and he also claims to have demonstrated the presence of a fluorescent metallic compound.

Würster,[64] in a discussion of the preceding results, subsequently pointed out that no information exists as to the identity of the substance responsible for the fluorescence, except that it does not appear in the fat, and is probably not carried by the protein matter.

Urobilin (see Gerngross and Schulz, *supra*, and p. 253) is therefore unlikely to be the cause of the fluorescence of milk, creatine, creatinine, xanthine, and hypoxanthine have no yellow fluorescence, and lactose also is eliminated since it has a blue fluorescence. The main evidence is in favour of the yellow-green colouring matter of milk as the carrier of fluorescence (see, however, Boober [207]), and this conclusion is supported by experiments on the effects of sunlight (p. 160), since the bleaching of the yellow colour is accompanied by a change in the colour of the fluorescence to blue (*cf.* O. Warburg and W. Christian).[219]

It may be that, as suggested by Bleyer and Kallmann, there are two colours in milk: (*a*) water-soluble and (*b*) lipoid- (and also fat-) soluble, in which case the latter (carotene type) probably has little influence on the fluorescence. The water-soluble constituent (lactochrome) is, however, more sensitive to bleaching by sunlight, and is probably the cause of the fluorescence.

Isolation of Lactochrome.—Würster therefore deproteinised rennet whey by heat, and saturated the resulting product with ammonium sulphate; after evaporation *in vacuo* and extraction of the pulpy mass with 96 per cent. alcohol and evaporation of the extract, a yellow colouring matter was obtained. It gave, however, a blue colour in ultra-violet light.

Following Bleyer and Kallmann (*supra*) fresh rennet whey was then evaporated *in vacuo*, when an intense yellow-green fluorescence resulted. The residue was digested with two volumes of 96 per cent. alcohol at 0° C. for 2 days, and the

separated protein was removed in the centrifuge. The liquid portion was filtered (when it had a yellow colour and fluorescence) and evaporated to a syrup, when the residue appeared yellow-green in ordinary light, and gave a yellow-green fluorescence. It was further purified by shaking three times with 80 per cent. alcohol for 2 hours, and evaporated *in vacuo*. The final syrupy residue was soluble in water and in alcohol, and its fluorescence changed to blue on exposure to light and also on raising the *p*H value by addition of sodium hydroxide. Rapid dialysis was used as a means of transferring the colour (and with it, the fluorescence) to distilled water, and treatment with animal charcoal also removed both colour and fluorescence. Bleyer and Kallmann (*vide supra*) showed lactochrome to have similar properties, and there appears to be little doubt that this is mainly responsible for the fluorescence.

J. Cvitl [148] has described the preparation of the yellow fluorescent compound by freezing, while K. G. Stern [149] has isolated the lactoflavin by Kuhn's method.[150] G. C. Supplee, S. Ansbacher and Bender [220] applied the fluorescence method to the determination of lactoflavin in milk, using Kuhn's [150] method for separating the lactoflavin. A simpler method is due to C. H. Whitnah and his co-workers,[221] who treat the milk with trichloroacetic acid, centrifuge, and neutralise the serum (to methyl orange), and then dilute the sample until it can be matched under the lamp against standard solutions containing 0·12 to 0·06 γ of flavin. The results are claimed to compare favourably with those obtained by other methods for the determination of lactoflavin (vitamin B_2).

S. M. Weisberg and I. Levin [222, 256] describe a method for estimating riboflavin (vitamin B_1), in which the sample is heated under reflux with acidified methyl alcohol in an atmosphere of carbon dioxide in the dark, diluted with acetone, cooled overnight at 0° C., and finally filtered and evaporated *in vacuo*. The sample is made up to a definite volume and compared against a calibrated solution of fluorescein. The unknown solution is diluted so that the range of concentration of riboflavin falls within the limits 0·1 to 1·0 γ per c.c. F. H. Cohen [223-4] and G. C. Supplee and co-workers [225] have also used the yellowish-green fluorescence for the determination of riboflavin, and G. N. Murthy,[226] using an adsorption technique, finds the results for 40 foods to agree

closely with the biological assay. Supplee and his co-workers [227] mention that most commercial caseins and a few "purified vitamin-free" caseins are contaminated with lactoflavin, the presence of which can be seen under the lamp.

G. Barger and co-workers,[245, 248] following up the work of Peters [246] and others,[247] on the reactions and constitution of Vitamin B_1 have deduced a structural formula which appears to fit the chemical and physical evidence.

Vitamin B_1 in urine has been estimated by the thiochrome reaction by J. Goudsmit,[249] by H. G. K. Westenbrinck,[249, 250] and by W. Karrer,[251] while M. Pyke [230, 252, 253] has investigated the value of Jansen's version [231] of the method for other materials, the fluorescence being measured photo-electrically. Good results were obtained by the former workers, but Pyke found that with certain foodstuffs the amount of vitamin given by the fluorescence method is lower than the biological assay. It would appear that if vitamin B_1 is added in the crystalline state or in a pure form it can be determined quantitatively, but in certain substances the vitamin is present as the pyrophosphate or perhaps, as recently suggested, in combination with proteins. In the former case, it is insoluble in *iso*butyl alcohol, and in the latter it is separable from the tissues only with difficulty, and consequently the results are not quantitative. Pyke quotes the case of yeast which contains a substance having a strong blue fluorescence which does not increase in intensity when the thiochrome reaction is carried out. In this case the fluorometric method would assess the vitamin B_1 present as nil, whereas by the biological method positive results are obtained. K. M. Henry, J. Houston and S. K. Kon [233] have also compared the two methods for raw and processed milks, but find that the fluorescence method does not provide a complete measure of vitamin B_1 activity in such cases, unless the sample is first incubated with a suitable enzyme at a *p*H value of 3·7 to 4·0.[261] Modifications of the thiochrome method [262] suitable for use with milks, milk powders, cereals, potatoes,[258] and for the determination of phosphorylated aneurin [259] and cocarboxylase [260] have also been evolved.

J. A. Radley [151, 152] examined several hundreds of samples from herds of cows giving Grade A, T.T. milk and the green-yellow fluorescent lactochrome separated by a modification of Wurster's method and found that, as with milk itself, the addition of

oxidising agents causes the fluorescence to become dull and finally to disappear; formaldehyde, however, preserved the colour indefinitely. Strong reducing agents caused the fluorescence to change to blue after varying periods of time ranging from 1 to 24 hours. The addition of butter-fat or of olive oil to the solution gives mixtures which act in the same manner as milk towards light and heat. Similar results were also obtained from milks reconstituted from spray-dried milks, but roller-dried milks gave a blue fluorescent compound.

The blue fluorescence developed by milk on standing, as noted by Baker and Taubes (p. 156), could not be obtained in bacteriologically pure milk, but in samples of unpasteurised unclean milks it was readily obtainable; their other observations were confirmed. It was also noted that pasteurisation did not affect the fluorescence colour, but after 30 minutes at 100° C. a decrease in the intensity of the fluorescence and a change in its colour towards white was produced (*cf.* G. Hermann).[228]

It would appear that the blue fluorescence shown by ordinary milks on standing is probably due to the reduction of the lactochrome to a compound having a blue fluorescence. This may take place in two stages, *viz.*, the reduction of the supply of available active oxygen by the bacteria of the milk, followed by a reduction of the lactochrome by some constituent of the milk, probably the fat. Shaking would thus partly restore the colour to yellow, but the blue fluorescent compound would gradually predominate until further shaking would have no further effect. The presence of bacteria and the supply of oxygen both appear to play an important part in the complex changes corresponding with the production of the blue fluorescence.

The fact mentioned above, that heated milk shows no fluorescence after ageing, has been confirmed by S. Rothenfusser,[153] and it is interesting to note that spray-dried milks are subjected to a lower temperature than roller-dried milks, and that they have a yellow and blue fluorescence respectively.

Kieferle mentions that whilst *fresh milk* has a yellow fluorescence, soured milk appears whitish to grey-violet, and some soured milk preparations, such as *Yoghurt*, have a white to yellow colour. Unsweetened *condensed milk* appears white with a tinge of blue. S. Kaloyéréas [229] detects the addition of goat's milk to cow's milk by adding equal volumes of a saturated brine solution

and of ether, when the fluorescence of the serum from cow's milk is blue, whereas that of goat's milk is yellow.

According to Schützler [56] the presence of certain metals in the curd modifies the fluorescence, *e.g.*, iron induces a bluish fluorescence.

Contamination of Milk.—F. M. Litterscheid [65] has devised a method to distinguish between external contamination of milk, and dirt carried in the milk itself. This is based on the fact that chlorophyll fluoresces red in filtered ultra-violet light (see p. 299). It is known that grass, hay and vegetable residues in cows' excreta do not fluoresce, or at the most, show only a faint, dark brown colour with a few patches of greenish or bluish fluorescence. If the residues of cows' excreta are coated with Japan lacquer, a bright raspberry-red fluorescence is obtained under the lamp, whilst dried albumin fluoresces from yellow to white, with a bluish tinge, and pus has a weak yellow colour. The appearance of milk residues, casein, albumin curds or pus is not affected by the varnish. J. Stark [154] has confirmed these results and points out that any solvent for chlorophyll would probably give the same result. This method might be of use for the routine examination of milk from farms, and the direct examination of milk under the lamp might also give useful indications whether the time of milking had been declared correctly.

The use of hypochlorites for disinfecting dairy plant other than mechanical milkers, is prohibited in England, and R. C. Wright and E. B. Anderson [232] find that 12 p.p.m. of chlorine in milk and coffee cream and 17 p.p.m. in full cream is detectable by means of the following test: 3 c.c. of milk cooled to 0 to 5° C. are mixed with an equal volume of 73·5 per cent. sulphuric acid containing 0·025 per cent. stannous chloride, which has been cooled similarly. The mixture is shaken while immersed in a freezing mixture, allowed to stand for 3 minutes, and then centrifuged and examined under the lamp, when a strong yellow fluorescence indicates the presence of an oxidising agent. The seat of the fluorescence appears to be the protein matter, as the fat, mineral or lactose contents do not affect the reaction.

Cream.—O. Mezger and his co-workers [66] have examined mixtures of an artificial cream and pure cream, and their results should prove of interest to those engaged in the examination of these products. Separated fat from *synthetic cream* appeared

pure white with a bluish luminescence, whilst genuine cream was a striking yellow colour. These colours are not modified by the addition to the creams of sugar, formalin or vanillin, but in the last case a number of dark bluish-grey spots appear. A much brighter yellow fluorescence is obtained with a mixture of true and synthetic cream, even in the presence of 50 per cent. of the latter. Radley and Gruber [151] have examined a number of creams and found that cream made from hardened fats invariably had a white fluorescence with a dull blue or violet tinge. Some reconstituted creams made from dried milk and butter were found to have a brilliant white colour, and it was found that the dried milks had been made by roller-drying, and that spray-dried milks always give cream with a yellow fluorescence.

J. Volmar [67] mentions the interesting fact that only cream that has been obtained from fresh milk fluoresces, and that this fluorescence decreases with ageing until eventually it disappears.

K. Braunsdorf [68] has examined homogeneous *natural coffee cream* and mentions its yellow fluorescence, the colour becoming paler as the fat content increases (*e.g.*, to 30 per cent.). It changes to white with a bluish tinge after ageing, or after heating at 65 to 70° C. for 1 to 24 hours.

Cheese.—In cheese-making the following changes may be observed :—

Milk before starter : yellow fluorescence.

Milk and starter : dull blue, yellow on surface ; all yellow on shaking but not so strong as originally.

Curd at setting : curd, blue ; after exposure to air, yellow.

Liquid—yellow at top, blue at bottom. On shaking in air, yellow.

Curd at cutting : daylight—three layers, *viz.*, top, clear ; opalescent watery layer ; curd. Under lamp—top layer, brilliant yellow ; watery layer, pale blue ; curd, deep blue.

Curd after whey has been drawn, after grinding and after salt : blue on fresh surfaces, turning yellow after exposure to air for 20 seconds.

This colour is maintained throughout all the stages of manufacture, although admission of air to the solid or liquid phase after the whey is drawn causes the return of the yellow colour.

The fungi of cheese show very intense fluorescence-effects which are often so strong that quite small colonies can be detected in the rind of soft cheeses such as Limburger or Brie. The living

fungi have a bright, light-green fluorescence which is not shown by decaying or dead fungi, and the method is therefore particularly suited to the examination of Roquefort and Gorgonzola. "False ripening" (for example, of Camembert cheese) can be detected by partial or complete failure to produce the fluorescence effect.

Further work on milk and cheese is described by D. Migliacci,[69] L. Vogeler,[70] T. Baumgärtel,[71] and by Iguchi and others.[72]

Eggs and Egg-Products.—The *white* of a hen's egg fluoresces when dried though not in the liquid state, whilst the white of a duck's egg has a distinct yellowish or bluish shimmer, and thus can easily be distinguished from vegetable albumin. The *yolk* of a hen's egg is feebly fluorescent, with a weak lemon colour, whilst the yolk of a duck's egg has a reddish-yellow fluorescence.

Since the fluorescence of eggs increases with time, *the age of eggs* may be determined. Hesselink was the first worker to note this, and his observation has since been confirmed by J. E. H. Waegeningh and J. E. Heesterman,[73] who have also introduced a method for the determination of the approximate age of eggs by comparison of the intensity of the fluorescence with a set of standards made from 0·1 to 12·5 per cent. solutions of gelatine. After 16 days the fluorescence of an egg is usually approximately equal to that of the 0·25 per cent. solution, and after 120 days it is found to match that of the 12·5 per cent. solution. C. Zach,[155] however, considers that the fluorescence of eggs depends often on the method of preservation and, therefore, that the estimation of their age in this way is uncertain.

Another method of estimating the age of eggs is due to Wehner[156] and to G. Gaggermeier[157] who have noted that the reddish fluorescence of egg-shell diminishes with time. For 10 days no change is apparent, but after this period the fluorescence changes through reddish-violet to blue. It must be borne in mind that the surface-treatment of the egg to preserve it might influence the fluorescence greatly; for example, A. Tapernoux[158] has actually used the lamp to differentiate treated eggs from fresh eggs, those preserved with lime-wash showing a very pale reddish-violet or a white fluorescence. The fluorescence spectra of egg-shells have been examined by H. Bierry and B. Gouzon.[159] Further work on eggs is due to A. Schrempf[160] and others.[161]

These changes in the fluorescence of the shell on ageing have been followed more closely by J. Straub and his co-workers.[234, 235]

The fluorescence of the shell, which is due mainly to porphyrin, is normally dull red when the egg is fresh, but gradually turns blue or disappears. The initial intensity varies considerably from one hen to another, and is associated with differences in the oöporphyrin content of the shell. The fluorescence of the shells of eggs from individual hens also is liable to regular changes, and the rate of change in fluorescence on storage appears to depend on the initial intensity of the fluorescence. Experiments with eggs from White Leghorns and from crosses between these and Minorcas, led to the conclusion that an appreciable quantity of eggs having a blue fluorescence in a batch of similar age and origin indicates that the eggs are at least 1 month old; on the other hand, the absence of blue eggs does not necessarily indicate that the batch is fresh. The inorganic phosphate content of egg-white also increases with the period of storage of the eggs. In this connection reference may also be made to the work of C. F. Van Oyen [238] on testing eggs.

It has previously been noted that organic dyes are, as a rule, highly fluorescent, and this fact has been used by T. Cockburn [74] and others to demonstrate the *removal of the mark of origin from eggs*. Further, the shell of an egg has, under the lamp, a uniform pink or purple tinge, but if the shell has had imprinted on it the place of origin, and if this has subsequently been removed by washing with water or with acid, then this spot stands out from the background as a dark patch; a case of this kind occurred recently in Scotland.[75]

Several workers [176, 177] have confirmed the usefulness of this method, although the colours recorded for the patches at the points of removal are not always the same, and range from red to blue. This might be expected if different dyes or different eradicators are used; acid eradicators, however, are unmistakable since they leave a patch with fluorescent edges. A. Schrempf [160] and also F. W. Vilter and O. Schmidt [161] have pointed out that eggs are sometimes cleansed by scouring, and that such eggs when viewed in ultra-violet light may convey the impression that a mark has been removed. Both K. Braunsdorf [178] and H. Mohler and J. Hartnagel,[179] therefore, recommend Heesterman's test [73, 180] in cases where direct observation gives doubtful results. The egg is soaked for 1 hour in a solution containing 0·5 c.c. of acetic acid and 1 c.c. of a saturated alcoholic solution of fuchsin per

litre, and is then washed with distilled water, dried and examined; thymol blue has also been used, and both reagents intensify the fluorescence of the affected area. A. R. Tankard,[244] however, describes experiments which seem to show that the washing of eggs in water produces no change in appearance under the ultra-violet lamp which might be mistaken for the effects of treatment such as would remove a mark of origin; in particular, treatment with acid shows a blotchy or patchy effect.

Preserved Eggs.—It has also been stated that eggs preserved in water-glass may be distinguished from unpreserved eggs by the modification in the fluorescence of the outside shell produced by this treatment. We (J. G.) have, however, been unable to confirm the existence of any such difference, and a more promising method of attack seems to be to paint the shell with a solution of an indicator which fluoresces in alkaline solution, when the treated egg might be distinguishable from the stronger alkalinity of its shell. Promising results have been obtained with iodo-eosin, but the method is not yet sufficiently advanced to enable further details to be given. According to Dingemans [76] the fluorescence of frozen eggs appears more reddish than that of fresh eggs.

Cocoa.—According to G. Popp cocoa containing husk shows no peculiarity under the ordinary mercury lamp, but R. Wasicky [77] and E. Gründsteidl,[78] who used a fluorescence microscope with a carbon arc as the source of ultra-violet light, state that the different portions of cocoa show different fluorescence colours, chiefly in shades of blue. Shell tissues have a buff or brown colour, mucilage cells appear colourless or else a light yellowish-green, whilst nib tissues show various shades from blue to violet. As is often the case, the finer the cocoa is ground the easier is the detection of the husk, and Wasicky considers that so little as 1·0 per cent. of this substance is detectable.

A *coffee substitute*, containing chicory and taraxacum root, was also examined microscopically by this worker,[79] the chicory being detectable by its yellowish-white fluorescence, and the taraxacum root by the blue colour. This method might be of great use in the detection of other berries and roots used occasionally to adulterate coffee.

Tea.—Although tea is frequently adulterated, there is little on record regarding the uses of fluorescence analysis in this connection. *Stachytarpheta*, which has been found in teas of Dutch

origin, is possibly an exception, although normally it is recognisable from the hairy upper surface of its stem and leaf. When, however, it is in a finely-divided state identification is less easy, and according to A. Steinmann [236] the fragments may be picked out by their bright violet fluorescence in ultra-violet light.

Preservatives.

Popp [47] mentions that the fluorescence of *boric acid* itself is yellowish-brown in colour, and that that of benzoic acid is light brown. J. Volmar,[67, 80] however, states that the fluorescence of benzoic acid is blue (which is correct), that of salicylic acid being violet, whilst the salts of both acids fluoresce with greater intensity than the free acids.

Shirov's work on the fluorescence of boric acid activated with fluorescein (see p. 198) might be of use for the detection of boric acid in fruits, jams, milk, creams, etc. A few samples of milk and cream containing about 0·01 per cent. have been examined in this way by one of us (J. A. R.) with fair success, but the possibilities of the method require to be investigated fully, especially with regard to the temperature of ashing and the mixing of the ash with the fluorescein.

So little preservative is required to preserve non-alcoholic wines, potted or dried meats and other foods, that its presence cannot be detected by direct observation, and the use of the lamp does not eliminate the necessity for the usual preliminary separation of the preservative. The lamp might be used, however, for the identification of the preservatives after the separation has been effected, and by the use of Danckwortt and Pfau's capillary-strip method a test of great delicacy could probably be evolved. On the other hand, the usual chemical tests are delicate and easily applied, and it is doubtful whether they could be replaced with any advantage.

The fluorescence of *sodium salicylate*, however, is so pronounced that one part of this compound can be detected in 25,000 parts of milk,[67] and under the best conditions one of us (J. G.) has found it possible to recognise half this quantity. The trade product sold under the name of " Abrastol " must first be decomposed into β-naphthol and potassium sulphate, made alkaline, and then examined under the lamp. G. Dejardin and

L. Herman [237] have examined the fluorescence of sodium salicylate from the spectroscopical point of view, and find that the fluorescence is best produced as the result of excitation by the mercury line 2482 Å.

The fluorescence of quinine in sulphuric acid has suggested to us (see J. Grant and J. H. W. Booth [128]) a method for the detection of small quantities of *sulphur dioxide* used as a preservative. A little of the sample is placed in a boiling-tube with 10 c.c. of water and a little hydrochloric acid, and the mixture is boiled gently so as to distil over about 3 c.c. into another tube containing 5 c.c. of water, 1 c.c. of pure, 40-volume hydrogen peroxide, and 0·5 c.c. of a saturated solution of quinine in water. Any sulphur dioxide is oxidised to sulphuric acid which causes quinine to fluoresce in the presence of ultra-violet light. A blank distillation should be carried out to ensure that no fluorescence is produced by "priming," and, of course, the original hydrogen peroxide should not be fluorescent. The maximum sensitiveness is about 0·25 mgrm. of SO_2. The method has also been extended to *sulphides* and *hydrogen sulphide* by Grant and Procter-Smith,[197] in which case 0·1 mgrm. of sulphide-sulphur is detectable.

Although not strictly in the category of "preservatives," the examination of crab soup for *artificial colouring matters* may be mentioned here, and further reference made to Chapter XIV. E. Baier and H. Barsch [170] have used the lamp for this purpose and claim that solutions of genuine crab soup fluoresce, but that if artificial colourings are used they remain dull. This, however, is not confirmed by the work of G. Büttner and A. Miermeister,[171] who found no difference between coloured and genuine soups. Of the colouring matters for foodstuffs which are prohibited in Britain, only naphthol yellow gives a green fluorescent lower layer in the following test (due to J. R. Nicholls).[254] A mixture of one volume of the alkaline sample, half a volume of strong sulphuric acid and a little solid potassium permanganate is boiled for 1 minute, and then just decolorised by addition, drop by drop, of sodium sulphite solution. A few crystals of resorcinol are then added, and the mixture is evaporated until acid fumes appear, when it is cooled, diluted and extracted with ether; the ether extract is then shaken with dilute ammonia. This procedure eliminates the possibility that a fluorescence may be

produced by the action of the excess of permanganate on the resorcinol.

The deterioration of *fish and meat products* owing to mould formation is discussed on p. 100 (Bacteriology).

Spices may also be conveniently considered under this heading, and reference may be made to pages 92 and 104 and to the work of E. Gründsteidl,[78] who describes the characteristics of a large number of such products when seen under the fluorescence microscope. The spices were examined in the form of powder, and in cross-section, but reference must be made to the original paper for full details. *Oil of pepper* appeared weak blue, and *pepper resin* yellow (grey-blue if pure). Apart from the distinction of *black* from *white pepper* (from the lighter shade of the husk particles in the latter case) the method appears too laborious, and the differences between the various spices too small to provide a very useful analytical method.

A. Juillet, A. Baussouls and J. Courp [81, 82] examined *mustard* and *oil of mustard* and substances used to adulterate the former, and consider that under suitable conditions falsification may be detected. Particles of talc or chalk, in particular, are detected by their lack of fluorescence. J. Grant and H. Procter-Smith have confirmed these results as a whole, although they found distinct differences between the colours of ground white and black peppers and corianders, these being white to yellow, brownish-grey with white spots, and a redder-brown, respectively. Ground pimento had no fluorescence, mace was golden-yellow, Jamaica ginger was cream, and nutmegs were dull-brown.

Mushrooms.—Edible and poisonous varieties are distinguishable (see p. 108).

Bakery Products.

Flour.—The examination of flour under the lamp has been carried out by G. Popp,[47] J. Grant [136, 213] (see p. 91), J. Volmar,[80] A. Karsten [200] and G. Capelli.[83, 84] The samples examined should all be in the same condition of humidity if comparative results are required. Sound *wheat* and *rye* flours show a clear bluish fluorescence, which changes to yellow on treatment with acids or with ageing,[131] whilst *barley* and *potato* flours appear white and greyish-brown respectively. *Pea* flour has a red fluorescence, and *bean* flour a bluish-green fluorescence. In the method of

J. Grosbüsch [199] an extract of the flour in 70 per cent. alcohol is spotted on to filter-paper, and the dry paper is examined in ultra-violet light. The respective colours before and after addition of Fehling's solution are intense green and yellow for rye, and none and red-blue for wheat. The method may be applied to bread, and 10 per cent. of rye flour is detectable.

By means of a microscope, so little as 2 per cent. of bean flour can be detected in admixture with wheat flour, and the finer the flour is ground the easier is the detection of the former. As previously mentioned (p. 163) the dried white of egg fluoresces, and addition of egg albumin to flour can therefore be detected (according to Volmar [67]) even if only two eggs have been added to one pound of flour. J. Tillmans and his co-workers [172] have repeated this work and consider that 2¼ eggs to one pound of flour gives a mixture in which the eggs are just detectable by the weak blue fluorescence they impart. Further work on such admixtures has been done by T. Kühlen,[85] and Barbade [131] and Pannier [173] have shown that the ordinary microscopic methods are greatly facilitated if ultra-violet light is used as the source of illumination (*cf.* p. 78). Thus, the latter found that if eosin is added to wheat flour a red-orange fluorescence is obtained, whilst blue mould spots appear sulphur-yellow; methylene blue produces no distinctive colour.

A luminous yellow-green fluorescence is obtained with flour made from *soya beans* from various sources, but if the oil of the bean has been extracted in a solvent, then a considerable weakening in intensity occurs, and the fluorescence often changes in colour to canary-yellow. In filtered ultra-violet light Italian flours containing 82 per cent. to 84 per cent. of wheat flour show a luminous, scaly (mother of pearl) effect, and a characteristic bluish-violet fluorescence. This fluorescence is diminished by the presence of 1 to 4 per cent. of soya bean flour, and in the presence of 10 per cent. is almost replaced by a canary-yellow colour; over 10 per cent. of the soya bean flour completes this change.

Capelli has also shown that the foreign and poisonous *seeds* and extraneous inorganic matter liable to be found in the wheat can often be detected from the fluorescence. *Vetch flour*, for instance, fluoresces with an orange-red colour, and the presence of so little as 2 per cent. in grain flour causes a change of the

bluish-violet tone to a more red colour. Capelli also mentions that addition of zinc oxide, which fluoresces with an intense reddish-yellow colour, can be detected even when it is present to the extent of only 0·3 per cent.

F. Albrecht [86] has made the interesting observation that seeds from grains of *barley* which appear to be of the same type fluoresce under the lamp in green, violet or blue ; and this is discussed further on pages 90 to 94, where will also be found an account of some interesting work carried out by S. P. Mercer and P. A. Lineham on Scottish and Irish ryes.

According to K. Seipel [87] *new wheat* is characterised by green fluorescent spots which disappear on ageing. The fluorescence varies with time and processing, and uniformity of colour is no actual proof that the corn is of high quality. According to Barbade [88] the cross-section of wheat which gives the best flour appears bluish, since it has a higher gluten content, whilst inferior samples appear more yellow. The intensity of fluorescence of various flours has been measured by means of a photo-electric cell by G. Berheim and M. Guyot.[89] White haricot bean flour gives an intensity of 14 on the arbitrary scale, red haricot 8, maize 4, pea 7 and lentils 5·5.

Further work on various flours has also been done by Volmar,[90] whilst Mogos [91] considers that the treatment of flour with nitrogen peroxide or with chlorine compounds may be detected by extraction of the flour with ether, the extract from treated flour having a much weaker fluorescence.

Seeds are discussed on p. 90 (Agriculture).

Fermented Liquids.[135]

Vinegar.—The fluorescence from 414 samples of vinegar, both manufactured and fermented, were investigated by H. Wüstenfeld and H. Kreipe,[92] whilst A. Janke and H. Lacroix [93] carried out similar investigations, and at the same time determined the iodine absorptions of the samples. These last two workers found that fermentation vinegar and essence vinegar can be differentiated by the degree of fluorescence in ultra-violet light, but consider that the iodine absorption value is more useful for the detection of admixture. The degree of intensity of the fluorescence is, however, not proportional to the

iodine value of the vinegar, and the substance giving rise to the fluorescence is considered to be a product of bacterial metabolism. A. R. Tankard [198] also mentions the use of the method to distinguish malt and artificial vinegars. By growing *B. Ascendens* in alcoholic solutions of mineral salts, Janke and Lacroix obtained a fluorescent liquid which had the power of absorption of iodine, and stimulated the growth of yeast cells. F. Flury [94] has shown that the fluorescent component has no connection with the presence of enzymes or of vitamins, as the fluorescence may still be observed in vinegars devoid of these.

Wine and Spiritous Liquors.—A great deal of work has been carried out on the examination of wine in ultra-violet rays, the samples being either examined directly, or pre-treated in one of a number of ways. *Red wine* placed under the lamp without previous treatment shows a deepening in colour which A. Hanak [95] has demonstrated to be due to the iron present. A typical white fluorescence, described by Litterscheid as a milky opalescent fluorescence, is shown by *white wine*. R. Moredod,[96] and also J. Werder and C. Zäch [97] have shown that wine made from dried grapes gives a blue fluorescence under the lamp, whilst normal wines give either a dark green coloration or else none at all. Pure *fruit wines*, on the other hand, give only a brown turbidity.

The *detection of fruit wines in grape wines* has been carried out by V. Reich and M. Haitinger,[98] and further, by F. M. Litterscheid.[99] The first investigators showed that all red wines examined under the lamp gave a dark colour throughout. They recommend shaking the wine with ether or chloroform, and then examining the ether extract, since in this way the fluorescence of the wine can be observed without appreciable interference by its colour. Alcohol should be removed from white wines, and wines rich in alcohol, by heating on the water-bath, and a sharper separation between the wine and the ether is thus obtained. Litterscheid shakes the wine with amyl alcohol, when the extracts from unadulterated white wines fluoresce with a white to whitish-green colour, whilst with all true apple and pear wines examined the amyl alcohol layer fluoresces with a bright azure-blue colour. From mixtures of 25 per cent. of fruit wine with 75 per cent. of white wine a clear, bluish fluorescence is obtained, and this can be compared with that given by a sample of pure white wine.

G. Reif [100] has examined extracts of wood in alcohol, acetic acid or in white wine distillates, the colour of which is due to substances extracted from the wood of the casks. He also examined *brandies* made from grape marc, from fruit, and from grain, and showed that there is almost complete correspondence between their tannin contents and the intensity of fluorescence. The fluorescence of certain wine distillates, brandies, arrack, and rum is also often influenced by other substances present in solution.

The behaviours of *alcoholic distillates of various brandies* derived from potato, apples, grapes, rye and maize have also been examined by M. Rüdiger and E. Mayr.[101] The procedure of these workers was to dilute the brandy until it contained 33 per cent. of alcohol by volume, and 100 c.c. of the liquid were then distilled, nine 10 c.c.-portions being collected and 10 c.c. retained as residue. These distillates and the residue were examined in ultra-violet light, and it was found that the fluorescence was most marked in the fourth fraction and in the residue, although the results for the different brands varied so little as to render the method useless for the detection of adulteration. On further dilution the maximum fluorescence was found to appear in an earlier fraction, and the addition to the brandy of an artificial essence gave distillates in which the fluorescence occurred in fractions later than the fourth. Oily droplets of a fatty acid nature were obtained between 94 and 97° C., and these also showed a strong fluorescence. The fluorescent substance contained in the fourth fraction was removable by precipitation of the distillate with silver nitrate solution. The method of H. Barsch [239] is on similar lines, the sample being distilled in a special rectifier and divided into 7 fractions ; the fraction boiling between 48° and 55° C. has a strong blue fluorescence if it is derived from brandy, although some rums also give a positive test.

M. Haitinger and V. Reich [108] have used capillary analysis (see p. 59) as a means of investigating grape wine. The portion of the strip immersed in the wine and the zone just above it fluoresce with a pink to yellow colour, and above these is a dull blue-violet zone, the upper half of the strips having a yellow to grey-green fluorescence. Fruit wines give violet zones, and the colour of mixtures is intermediate between those of the constituents. K. Müller, E. Vogt and A. Raesch,[102] C. von der

Heide,[175] and B. Bleyer and W. Diemair,[103] however, express some doubt about this method, as it does not allow differentiation between grape wines and fruit wines treated with eponit (active charcoal). Furthermore, differentiation is not clear between red wine and a mixture of red wine and white wine, or between hybrid white wine, and wine made from grape marc or dried grapes.[211] Examination of an extract in amyl alcohol may possibly provide a means of enabling this differentiation to be made.

It is therefore advisable (after A. Heiduschka and E. Mohlau,[104] as well as J. Werder and C. Zäch [97]) that the wine to be examined should first be decolorised with animal charcoal (not more than 0·25 grm. of charcoal for 12 c.c. of wine), and the fluorescence of the wine then compared with artificial standards. A. Röhling and J. Richarz [162] also used active charcoal (" sorbit "), but they examined the adsorption complex which normally has a yellow-green fluorescence, although in the presence of mannite the tribenzyl mannite first formed changes it to dark violet (see also A. Miermeister and F. Battay [163]). Werder and Zäch [97, 105] used mixtures of glycerin and water, whilst Heiduschka and Mohlau employed sodium saccharate dissolved in ethyl alcohol. The latter has the advantages that the solution does not show any change in fluorescence even after 4 months, that it is not hygroscopic, and that the sodium salt can easily be weighed accurately.

P. Berg and his co-workers [106, 107] consider that the carbon with which the wines are shaken removes the substances which inhibit fluorescence as well as the colouring matter, but their observations are nevertheless in accord with those of other workers. The glycerin-water standards mentioned above are divided by Werder and Zäch into six classes, the first corresponding with a pure natural wine, and the last with a wine made from dried fruit; obviously the most difficult class to assess with accuracy is that which shows an intensity corresponding with standard number 3. Greek, Sicilian and Samnian wines [105] all gave positive fruit juice reactions by this method, and it may be that owing to the warm southern weather the grape crop contained dried fruit which had been pressed with it. Since such " natural " fruit wines show this, the value of the method is somewhat diminished.

The source of the fluorescence of wines has been investigated by Haitinger and Reich,[108] who endeavoured to ascertain to which

part of the fruit it is due. The fruit was therefore examined whole or dissected under the lamp, and an impression of the cut-section was also made on a sheet of filter-paper, and this impression examined in filtered ultra-violet light. A number of fruits were examined in this way, including different varieties of grape, carrot, parsley, onion, and also the sap of the milk-weed (spurge, blue-violet). The juice of the celandine gave an intense golden-yellow fluorescence. J. Khouri [181] attributes the fluorescence of dry grape wines to complex carbohydrates and resins. This branch of the subject requires further systematic investigation, and an important step has been made by L. Genevois [205] who considers that luminoflavin, the photo-derivative of flavin, is responsible for the natural fluorescence of urine, the flavin being converted into the photo-compound on exposure. Luminoflavin may be extracted in trichloroethylene and then has a blue fluorescence which disappears when the *p*H value is brought above 8 by addition of alkali.

J. Kloss and W. Seifert [109] have used the method for the detection of *gallic acid* in fruit and grape wines, since the ether extract of this acid fluoresces blue in ultra-violet light. The titration of wines in ultra-violet light is dealt with on page 315, and further work on this subject is due to Volmar and Clavera.[113]

T. Koana [110] has examined *brewery products* under the lamp, and V. Bermann [111, 112] and co-workers have also worked on the fluorescence of malt and beer. The latter shows a faint green to bluish-green fluorescence, and Bermann [111] holds the opinion that this is due neither to mineral constituents nor to the products of hydrolysis of starch or protein. S. Pickholz [182] found that although pale fine barleys differ from dark, old or coarse barley, the method offers no advantages for the differentiation of old corns (*cf.* p. 91); the colours are modified by steeping to an extent which depends on the *p*H value of the water used. Colours due to caramel or sugar are easily distinguished from those natural to the malt, and brewer's pitch and added colouring matters may also be recognised (see J. Grant [213]).

L. Genevois and L'Espil [206] were able to extract a fluorescent flavin from *yeast* and to show that the fluorescence is not inhibited by the action of formaldehyde on the $—NH_2$ group, or by oxidation of the —SH group with iodine. Pett [212] deals with the fluorescent products of yeast metabolism.

Confectionery.

Sugar.—Popp (*loc. cit.*), among others, has also studied the fluorescence of sugars in ultra-violet light, and S. Judd Lewis has measured the intensity of such fluorescence with the apparatus described on page 341. Beet sugar has little or no fluorescence, but lactose, crude cane sugar, and solid glucose appear reddish under the lamp. As a rule sucrose shows no fluorescence, although glucose syrup lights up with a bluish glow. Lewis [257] believes that any observations on the fluorescence of sugars should be judged very critically, unless the sample has been rigorously purified.

According to K. Sándera [114] the natural fluorescence of *sugar-factory products* is due to a colourless, or slightly-coloured substance, which is one of the first products of the caramelisation of sugar, or of the destruction of invert sugar. It is soluble in ether and chloroform, and does not seem to be connected in any way with the ash or the colouring matter of the sugar. Mezzadroli and Vareton [115] have investigated sugar-products at all stages from the raw to the finished material, but no details as to the colours of the fluorescence are given. It is claimed that differentiation is possible between sugars refined with animal or vegetable carbon, and that to a certain extent it is possible to identify the plant in the latter case, but this statement should be accepted with great reserve. Invert sugar, however, is certainly detectable in many mixtures. Mezzadroli and Vareton [166] have also worked out a method for the differentiation of artificial and natural *lemonades and syrups*.

H. Lundén [116] has examined the fluorescence from a number of *commercial sugars* in the solid state and in solution by means of a frame containing several colour-filters (Fig. 11, p. 57). No fluorescence is shown by pure sugars with an ash-content of about 0·003 per cent., but the sugar assumes a dark violet colour which increases in depth on the addition of any non-fluorescent colouring matter. The addition of known amounts of such impurities to sugars, therefore, places at the disposal of the analyst a colour scale by means of which an estimation of the impurities in samples may be made. The method is claimed to be fairly delicate, a difference in ash-content of a fraction of a per cent. being shown by a distinct change in the fluorescence.

The greatest differences, however, are shown by the refined white sugars, whilst the addition of blue colouring matters has but little influence on the appearance in ultra-violet light. Impure raw sugar has a yellow fluorescence and some coloured sugars, *e.g.*, coarse loaf sugar, etc., appear almost white, and the purer the sugar becomes the more pronounced is the violet tinge mentioned above. These results should be accepted with reserve, as O. Spengler,[117] an accepted authority on such matters, has also worked on this subject but has failed to confirm them. It seems that this is one of the cases where the origin of the product is an important factor. In a later paper Lundén [165] gives the fluorescence of a number of sugar products together with data on hydrogen-ion concentration, ash-content, and spectrographic data, and further work on molasses and sugar is due to P. Bruère [118] and others.[137, 138, 255]

A number of the well-known *qualitative tests for sugars* are rendered more sensitive if carried out in ultra-violet light. Thus :—

Dextrose.—(1) A solution in 5 per cent. sodium hydroxide produces no fluorescence, but after 5 minutes on the water-bath a green colour is apparent. If a solution in 10 per cent. hydrochloric acid is used no colour is apparent even after 5 hours on the water-bath, although it appears if the solution is then made alkaline.[184, 185] The colour is blue-green at pH 7 and yellow-green at pH 7·5, and it may be used for the detection of glycosides. (2) A solution of β-naphthol in alcohol produces a green fluorescence.[185, 186] (3) Hydrochloric acid and a 1 per cent. solution of orcinol produce a yellow-red colour which dissolves in alcohol and then shows a green fluorescence.[185, 186]

Laevulose, Arabinose and Rhamnose.—A green fluorescence is obtained by warming with a solution of zirconium oxychloride (the Rimbach-Weber reaction) ; polyhydric alcohol sugars (*e.g.*, mannite and dulcite) do not react.

Aldoses and Methyl Pentoses are heated with hydrochloric acid and naphthoresorcinol, when a compound results which produces a green fluorescence when dissolved in alcohol (the Tollens-Rorive reaction).

Fructose (2 parts) is warmed with equal parts of resorcinol and hydrochloric acid (sp. gr. 1·18) and the resulting red precipitate is dissolved in alcohol, sodium carbonate is added, and

the orange-red solution extracted with amyl alcohol; a red to green fluorescence results.[185, 186] The combined influence of high temperature and ultra-violet light is to produce a yellow colour and fluorescence, which according to R. Cantieri,[240] is probably due to the formic acid produced by oxidation of the formaldehyde liberated by the action of the radiations.

Sucrose.—J. A. Radley has found that in cases of doubt with Cayaux's method for the detection of sucrose in cream or milk, the lamp may be of great assistance. To 15 c.c. of the milk are added 0·1 grm. of resorcinol and 1 c.c. of concentrated hydrochloric acid, and the mixture is heated in boiling water. In the presence of sucrose a red coloration is obtained, pure milk giving a brownish colour. When little sucrose is present the colour is sometimes difficult to detect, but examination under the lamp shows the fat layer to have an intense pale lilac fluorescence similar to that of some mineral oils, whilst a control has the usual yellow fluorescence. The intensity of the fluorescence corresponds approximately with the amount of sucrose present. Sucrose may also be detected by means of its reaction with boric acid (see p. 198). A. Amati and G. Bolzano[243] examined the principal sugars both in the crystalline state and dissolved in water, alcohol or ether, and believe that the differences observed serve as a means of identification. In general, however, there is little difference between the appearance of the sugar in the crystalline state and when dissolved in water, and it seems unlikely that the method will have many applications other than to the identification of pure substances. An exception perhaps is the detection of *invert sugar* in sucrose, because the blue fluorescence of the pure sucrose is displaced by a milky yellow tinge to an extent which depends on the amount of invert sugar present. These applications of the method and its uses on the manufacturing side of the sugar industry are dealt with further by M. Déribéré.[242]

Dulcin in the presence of saccharin (see also *Saccharin*, below).—In the method of H. J. Vlezenbeek[241] 10 mgrms. of sample are heated for 2 minutes with 10 mgrms. of resorcinol and 2 c.c. of strong sulphuric acid at 180° C., and the red solution is cooled and divided into 2 portions; these are made alkaline with ammonia and sodium hydroxide when a red-brown fluorescence obtained immediately, and a violet fluorescence after 10 minutes,

respectively. Oxidising agents destroy the fluorescence. The sensitiveness is 1 per cent. of dulcin in the presence of saccharin.

Glycerol.—H. Mohler and H. Benz [167] have elaborated a test which can be applied to its detection in certain foodstuffs *e.g.*, in marzipan. The substance is repeatedly extracted with ethyl alcohol and lime, the extracts being evaporated. Finally the residue is extracted with absolute alcohol, the alcohol is removed by evaporation and 10 c.c. of fresh 0·5 per cent. bromine water are added. The glycerol is thus oxidised to dihydroxyacetone and the bromine is removed by heating for 20 minutes on the water bath; 0·1 to 0·4 c.c. of a 2 per cent. solution of β-naphthol in alcohol and 2 c.c. of concentrated sulphuric acid are then added and the mixture is warmed for 2 minutes. A yellow-green fluorescence is obtained in the presence of 1 per cent. glycerol.

If *saccharin* is heated with resorcinol and strong sulphuric acid, and the mixture is then diluted and made alkaline, a weak orange-red colour having an intense green fluorescence and due to sulphofluorescein is produced (Börstein's reaction).[241] The sensitiveness is 1 mgrm. of saccharin in 6 litres of water, but the reaction is also given by barbituric, *o*-phthalic and other related acids (see also *Dulcin*, above).

Volmar mentions that saccharin has a whitish glow, but that if it is made alkaline with sodium hydroxide solution an intense bright violet fluorescence develops.

Jams, Marmalades and Pulps.—Marmalade appears dirty and discoloured, and according to Litterscheid,[119] pure apple pulp shows no special fluorescence effects, but only a dark brown colour. Apple pulp containing sucrose has a bright brownish-grey coloration, turnip pulp gives it a slate-coloured fluorescence, whilst addition of molasses produces a bright bluish-green colour; commercial glucose has a bright azure-blue colour.

The technique recommended is to precipitate a 2·5 per cent. solution of the pulp with an excess of lead acetate and to examine the liquid after filtration. Pure apple pulp then gives a dark brown fluorescence and an ochre-yellow colour on the surface; molasses show a reddish-grey, turnip pulp filtrate a bright green, and apple pulp containing sucrose a dirty yellow fluorescence. In this way the presence of 10 to 20 per cent. of turnip pulp, or more than 10 per cent. of molasses, can be detected in apple pulp.

The detection of apple pulp in jams or fruit-jellies is based on the observation of C. F. Muttelet,[120] that gooseberries, raspberries, strawberries and currants do not contain malic acid, but that this acid is invariably present in apples.

The presence of malic acid in jams supposed to be made exclusively from these fruits would therefore be strong presumptive evidence of the addition of apple pulp or jelly. L. Colombier [121] has suggested a fluorescence test for malic acid, sensitive to so little as 0·01 mgrm., in which 3 c.c. of the liquid are placed on a watch glass, 1 c.c. of a 0·1 per cent. solution of resorcinol is added, and the whole is evaporated on the water-bath. The residue is dissolved in 1 c.c. of 1 : 2 sulphuric acid and heated for 10 minutes on the water-bath, after which 10 c.c. of water are added and the liquid is neutralised with sodium hydroxide solution. The presence of malic acid is shown by a bright blue fluorescence in ultra-violet light. A similar test in which orcinol replaces resorcinol is described by S. A. Celsi,[122] who considers that this test is more specific since resorcinol also reacts with citric and aceto-acetic acids. Another, but less specific test is given on page 309.

Dicarboxy- and keto-acids give characteristic colours if heated with resorcinol and sulphuric acid.[208]

Honey.—When honey is examined under the lamp, either in solution or in a mass, no particular fluorescence is observed, but by means of the capillary method most natural (but not artificial) honeys have been found to give a light blue zone topped by a white zone. G. Orbán and J. Stitz [123] divide these colours into six grades according to their absorption spectra. They found that on evaporating some of the water from honey the intensity of the fluorescence was increased, but in greater proportion than can be explained by mere concentration, and furthermore, that the intensity appears to be a function of the viscosity. In thin layers slightly caramelised honey fluoresces more strongly than normal honey, but if it has been strongly caramelised then the fluorescence is weaker (*cf.* Sándera, *supra*).

A rough indication of the *origin of honeys* can be obtained from a determination of the fluorescence colours and the absorption spectra of various brands, and although it is difficult to distinguish natural from artificial honeys, yet adulteration is at once apparent. The absorption of six varieties of honey in the

ultra-violet region has been studied by Stitz and J. Koczkás,[1] who obtained characteristic absorption curves with sharp maxima at 2700Å.

Further references to foods may be found in the Bibliography,[125-135] and in Chapter II.

Essential Oils.—The influence of constituents, methods of extraction, age and origin are discussed by E. Ekman and A. Samyschlayeva,[130] and on page 302.

REFERENCES.

1 R. Marcille, *Ann. Falsif.*, 1928, **21**, 189.
2 A. Baud and Courtois, *ibid.*, 1927, **20**, 574 ; *J. Pharm. Chim.*, 1928, **120**, 215.
3 A. Baud and Courtois, *Ann. Chim. analyt. applic.*, 1928 (2), 10, 11.
4 S. Musher and C. E. Willoughby, *Oil and Fat Industry*, 1929, **6**, 15 ; and Nos. 12, 19 and 33.
5 Frehse, *Ann. Falsif.*, 1925, **18**, 204.
6 R. Stratta and A. Mangini, *Giorn. chim. ind. appl.*, 1928, **10**, 205.
7 A. le R. Glantz, *Jour. Ind. Eng. Chem.* (*Anal.*), 1930, **2**, 256.
8 E. R. Bolton, *Analyst*, 1930, **55**, 746.
9 A. G. Nasini and P. de Cori, *Ann. Chim. appl.*, 1929, **19**, 46.
10 A. van Raalte, *Chem. Weekblad*, 1928, **25**, 544 ; *Zeit. Unters. Lebensm.*, 28, **56**, 195.
11 J. A. Pierce, *Zeit. Unters. Lebensm.*, 1930, **59**, 94.
12 J. A. Pierce, *Johns Hopkins Univ.*, 1930, Aug. 6.
13 G. Loew, *Ind. olii min. grassi.*, 1929, **9**, 184.
14 G. Loew, *ibid.*, 1930, **10**, 140.
15 H. Marcelet, *Compt. rend.*, 1930, **190**, 1120.
16 H. Marcelet and H. Debono, *ibid.*, 1930, **190**, 1552.
17 E. Lewkowitsch, *Jour. Soc. Chem. Ind.*, 1927, **46**, 195T.
18 Bureau of Standards, *Jour. Franklin Inst.*, 1927, **204**, 805.
19 F. Croner, *Zeit. angew. Chem.*, 1926, **39**, 1032.
20 Forjaz, *Rev. Chim. Pura Applic.*, 1930, **5**, 29 ; *Brit. Chem. Abs.*, *B.*, 1931, 983.
21 H. Marcelet, *Compt. rend.*, 1928, **186**, 226.
22 C. A. Morehouse, *Proc. Iowa Acad. Sci.*, 1929, **36**, 297.
23 J. F. Carrière, *Chem. Weekblad*, 1928, **25**, 632.
24 H. P. Stadler, *Zeit. Unters. Lebensm.*, 1928, **55**, 404.
25 M. Haitinger, H. Jörg and V. Reich, *Zeit. angew. Chem.*, 1928, **41**, 815.
26 R. S. Morgan and K. MacLennan, *Biochem. Jour.*, 1928, **22**, 1514.
27 P. R. Peacock, *Lancet*, 1926, **211**, 328.
28 E. Nováček and J. Hökl, *Tschechnish. Zvěrol. Rozpravy*, 1930, 4.
29 E. Nováček and J. Hökl, *Zeit. Fleisch. Milchhyg.*, 1931, **12**, 47.
30 O. Laxa, *Nahrungsmittelindustrie*, 1930, **9**, 231.
31 J. Lenfeld, *Zeit. Fleisch. Milchhyg.*, 1931, **11**, 243.
32 J. Lenfeld, *ibid.*, 1929, **39**, 451, 454, 477, 482.
33 J. Lenfeld, *Tschechnish. Zvěrol. Rozpravy*, 1930, 4.
34 J. Lenfeld and E. Nováček, *Zeit. Fleisch. Milchhyg.*, 1929, **39**, 387.
35 J. Lenfeld, *Sonderdruck. Cs. A. Z.*, 1930 (*Tschechnish*).

[36] O. Gerngross and M. Schulz, *Chem. Ztg.*, 1927, **51**, 501.
[37] J. Hökl, *Zeit. Unters. Lebensm.*, 1932, **63**, 444.
[38] A. van Raalte, *Chem. Weekblad*, 1926, **23**, 580.
[39] A. van Druten, *Zeit. Unters. Lebensm.*, 1929, **57**, 60.
[40] A. van Raalte and A. van Druten, *Chem. Weekblad*, 1929, **26**, 602.
[41] F. Weiss, *Zeit. Unters. Lebensm.*, 1928, **56**, 341.
[42] K. Braunsdorf, *ibid.*, 1932, **63**, 308, 407.
[43] A. van Raalte, *ibid.*, 1927, **54**, 321.
[44] E. Feder and L. Rath, *ibid.*, 1927, **54**, 321.
[45] Gröning, *Zeit. Fleisch. Milchhyg.*, 1929, **9**, 153.
[46] J. Grossfeld, *Zeit. Unters. Lebensm.*, 1928, **55**, 376.
[47] G. Popp, *ibid.*, 1926, **52**, 165 ; *Chem. Ztg.*, 1926, **50**, 588.
[48] A. Knapp, J. E. Moss and A. Melley, *Analyst*, 1927, **52**, 452.
[49] J. Ripert, *Ann. Falsif.*, 1929, **22**, 459.
[50] W. Schmandt, *Zeit. angew. Chem.*, 1929, **42**, 1039.
[51] W. T. Field, *Analyst*, 1930, **55**, 745.
[52] W. Sproesser, *Chem. Umschau Fette, Oele, Wachse, Harze*, 1928, **35**, 325.
[53] H. P. Kaufmann, *Zeit. angew. Chem.*, 1928, **41**, 1123.
[54] J. D. van Roon, *Chem. Weekblad*, 1929, **26**, 576.
[55] G. W. Baker, *Analyst*, 1932, **57**, 38.
[56] K. Schützler, *Milchwirtsch. Forschungen*, 1927, **4**, 352.
[57] Kieferle, *Suddeutsche Molkerei Ztg.*, 1928, Dec. 13, 50.
[58] O. Gerngross and M. Schulz, *Milchwirtsch. Forschungen*, 1928, **6**, 567.
[59] G. W. Baker, *Analyst*, 1932, **57**, 38.
[60] J. Spolverini, *Riv. Ital. di Actinologia*, 1927, **2**, No. 9.
[61] J. Spolverini, *ibid.*, 1927, **2**, 1.
[62] F. Tallo and B. Albanese, *Riv. Sanitaria Siciliana*, 1930, Vol. 18.
[63] C. Schuetzler, *Milchwirtsch. Forschungen*, 1928, **6**. 883.
[64] K. Würster, *Zeit. Fleisch. Milchhyg.*, 1930, **11**, 379.
[65] F. M. Litterscheid, *Zeit. Unters. Lebensm.*, 1927, **53**, 263.
[66] O. Mezger, A. Schrempf, and M. Volkmann, *Suddeutsche Molkerei Ztg.*, 1929, Sept. 26, 1360.
[67] J. Volmar, *Jour. Pharm. Chim.*, 1927, **5**, 435.
[68] K. Braunsdorf, *Zeit. Unters. Lebensm.*, 1932, **63**, 308.
[69] D. Migliacci, *Boll. Chim. farmac.*, 1928, **67**, 673.
[70] L. Vogeler, *Molkerei Ztg.*, 1930, **44**, 1797.
[71] T. Baumgärtel, *ibid.*, 1931, **45**, 141.
[72] Iguchi, Kenzo, and Kentara Mitamura, *Milchkontrolle*, 1929, **2**, 151.
[73] J. E. H. van Waegeningh and J. E. Heesterman, *Chem. Weekblad*, 1927, **24**, 622 ; 1932, **29**, 134, 138, 650.
[74] T. Cockburn, *Analyst*, 1930, **55**, 42.
[75] *The Glasgow Bulletin*, Oct. 11, 1929.
[76] J. J. Dingemans, *Chem. Weekblad*, 1931, **28**, 350 ; 1932, **29**, 138.
[77] R. Wasicky and C. Wimmer, *Zeit. Unters. Nahr-. u. Genussm.*, 1915, **30**, 25.
[78] E. Grünsteidl, *Zeit. Unters. Lebensm*, 1932, **63**, 425.
[79] R. Wasicky, *Pharm. Post.*, 1913, **46**, 877.
[80] J. Volmar, *Bull. Soc. Chim. France*, 1926, **39**, 368.
[81] A. Juillet, A. Baussouls and J. Courp, *Bull. Soc. Pharm.*, 1931, **38**, 473.
[82] A. Juillet and J. Courp, *ibid.*, 1931, **38**, 562.
[83] G. Capelli, *Annali Chim. applic.*, 1927, **17**, 308.
[84] G. Capelli, *ibid.*, 1927, **17**, 513.
[85] T. Kühlen, *Chem. Tech. Rundschau*, 1929, **44**, 602.
[86] F. Albrecht, *Woch. Brau.*, 1927, **44**, 459.

[87] K. Seipel, *Zeit. ges. Getreidewesen*, 1930, **17**, 82.

[88] P. Barbade, *Ann. Falsif.*, 1931, **24**, 581 ; 1932, **25**, 468.

[89] G. Berheim and M. Guyot, "Traité d'Analyses par les Rayons Ultra-Violets Filtrés" (Paris : N. Maloine, 1932).

[90] J. Volmar, *Bull. Soc. Chim. France*, 1927, **42**, 302.

[91] M. Mogos, *Thesis* (Univ. Pharm., Paris, 1931).

[92] H. Wüstenfeld and H. Kreipe, *Deut. Essigindustrie*, 1928, **32**, 345.

[93] A. Janke and H. Lacroix, *Biochem. Zeit.*, 1929, **215**, 460.

[94] F. Flury, *ibid.*, 1929, **215**, 422.

[95] A. Hanak, *Zeit. Unters. Lebensm.*, 1930, **60**, 291.

[96] R. Moredod, *Ann. Falsif.*, 1929, **22**, 524.

[97] J. Werder and C. Zäch, *Mitt. Lebensmittelunters. Hyg.*, 1928, **19**, 60 ; 1928, **19**, 2.

[98] V. Reich and M. Haitinger, *Babofestschrift. Klosterneuburg*, 1927 ; *Allgem. Weinzeit.*, 1927, 44, No. 18 ; 1927, 44, Nos. 6 and 7.

[99] F. Litterscheid, *Zeit. Unters. Lebensm.*, 1927, **54**, 294.

[100] G. Reif, *ibid.*, 1929, **57**, 269.

[101] M. Rüdiger and E. Mayr, *ibid.*, 1929, **57**, 561.

[102] K. Müller, E. Vogt and A. Raesch, *ibid.*, 1927, **53**, 331.

[103] B. Bleyer and W. Diemair, *Chem. Ztg.*, 1929, **53**, 621, 641.

[104] A. Heiduschka and E. Mohlau, *Pharm. Zentr.*, 1930, **71**, 689.

[105] J. Werder and C. Zäch, *Deut. Weinblatt*, 1929, **10**, 111.

[106] P. Berg and E. Kroger, *Wein u. Rebe*, 1930, **12**, 327.

[107] P. Berg and L. Stockert, *Zeit. Unters. Lebensm.*, 1929, **57**, 448.

[108] M. Haitinger and V. Reich, *Allg. Wein-Ztg.*, 1927, No. 22.

[109] J. Kloss and W. Seifert, *Oesterr. Chem. Ztg.*, 1927, **30**, 117.

[110] T. Koana, *Bull. Res. Inst. Ferm. Japan*, 1929, **102**, 85.

[111] V. Bermann, *Woch. Brau.*, 1930, **47**, 215.

[112] V. Bermann and W. Breiss, *Zeit. ges. Brauwesen*, 1930, **53**, 53.

[113] Y. Volmar and M. J. Clavera, *Anal. Fís. Quím.*, 1931, **29**, 24; *Brit. Chem. Abs., B.*, 1931, 693.

[114] K. Sándera, *Zeit. Zuckerind. Czechoslav.*, 1927, **51**, 237.

[115] G. Mezzadroli and E. Vareton, *Atti* 111 ; Cong. Nazionale Chim. pura e appl. (Firenze e Toscana), 1929, 580.

[116] H. Lundén, *Zeit. angew. Chem.*, 1928, **41**, 199.

[117] O. Spengler, *ibid.*, 1928, **41**, 194.

[118] P. Bruère, *Ann. Falsif.*, Jan., 1932.

[119] F. M. Litterscheid, *Zeit. Unters. Lebensm.*, 1928, **55**, 175.

[120] C. F. Muttelet, *Ann. Falsif.*, 1926, **19**, 235.

[121] L. Colombier, *ibid.*, 1931, **24**, 89.

[122] S. A. Celsi, *Quim. e Ind.*, 1926, **3**, 205.

[123] G. Orbán and J. Stitz, *Zeit. Unters. Lebensm.*, 1928, **56**, 467.

[124] J. Stitz and J. Koczkás, *ibid.*, 1930, **60**, 420.

[125] T. Kühlein, *Chem. Tech. Rundschau u. anz. der Chem. Ind.*, 1929, **44**, 602.

[126] J. Volmar, *Bull. Soc. Chim. France*, 1927, **42**, 302.

[127] W. Ekhard, *Zeit. Spiritusind.*, 1927, **50**, 4.

[128] J. Grant and J. H. W. Booth, *Analyst*, 1932, **57**, 514.

[129] G. W. Baker and S. Taubes, *ibid.*, 1932, **57**, 375.

[130] E. Ekman and A. Samyschlayeva, *Riechstoffind.*, 1931, **6**, 140.

[131] P. Barbade, *Ann. Falsif.*, 1931, **24**, 581.

[132] M. Gisondi, *Ann. R. Staz. Chim. Agrara Spermi.*, 1931, Publ. No. 276 ; through *Brit. Chem. Abst., B.*, 1932, 1090.

[133] M. Auerbach, *Collegium*, 1932, **8**, 25.

[134] E. A. Mauersberger, *Chem. Ztg.*, 1932, **56**, 861.
[135] J. Grant, *Wine and Spirit Trade Rev.*, 1933, **62**, 150.
[136] J. Grant, *The Miller*, 1933, **59**, 309.
[137] A. Brieghel-Müller, *Z. Ver. Deut. Zuckerind.*, 1932, **82**, 141.
[138] G. Gérard, *Ann. Falsif.*, 1932, **25**, 212.
[139] G. Lunde and F. Stiebel, *Zeit. angew. Chem.*, 1933, **46**, 243.
[140] G. Lunde and F. Stiebel, "Fluoreszenz-Messungen an Olivenölen" (Oslo : Dybwad in Komm).
[141] T. Cocking and S. K. Crews, *Pharm. Jour.*, 1934, **133**, 86 ; *Quart. Jour. Pharm.*, 1934, **7**, 531.
[142] A. Bömer, "Handbuch der Lebensmittel Chemie," Vol. 2, Pt. 1 (Berlin : J. Springer, 1933).
[143] A. Wagner, *Chem. Ztg.*, 1929, **53**, 398.
[144] G. Lunde, H. Kringstad and H. W. Weedon, *Zeit. angew. Chem.*, 1933, **46**, 796.
[145] L. Francesconi and L. Pinoncelli, *Ann. Chim. Applic.*, 1934, **24**, 242.
[146] A. P. Forjaz, *Chim. e. Ind.*, 1932, **27**, 164.
[147] R. Nesini, *Zeit. Unters. Lebensm.*, 1934, **67**, 192.
[148] J. Cvitl, *Milchwirtsch. Forsch.*, 1931, **12**, 428.
[149] K. G. Stern, *Ber.*, 1933, **66**, 555 ; *Nature*, 1933, **132**, 784 ; 1934, **133**, 178.
[150] A. Kuhn, *Ber.*, 1933, **66**, 317, 576, 1034.
[151] J. A. Radley, *Analyst*, 1933, **58**, 527.
[152] J. A. Radley, "Fluorescence Analysis in Ultra Violet Light," Diss., London, 1933.
[153] S. Rothenfusser, *Zeit. Unters. Lebensm.*, 1930, **60**, 94.
[154] J. Stark, *Alpenländ Molkerei- u. Käserei-Ztg.*, 1931, **5**, 4.
[155] C. Zäch, *Mitt. Lebensm. Hyg.*, 1929, **20**, 209.
[156] Wehner, Deut. Landswirt. *Geflügel-Ztg.*, 1930, No. 11.
[157] G. Gaggermeier, *Arbb. Reichsgesundheitsamt.*, 1932, **65**, 105, 221.
[158] A. Tapernoux, *Compt. rend.*, 1932, **194**, 653.
[159] H. Bierry and B. Gouzon, *ibid.*, 1932, **194**, 653.
[160] A. Schrempf, *Zeit. Unters. Lebensm.*, 1933, **65**, 647.
[161] F. W. Vilter and O. Schmidt, *ibid.*, 1933, **65**, 649.
[162] A. Röhling and J. Richarz, *Chem. Ztg.*, 1930, **54**, 61.
[163] A. Miermeister and F. Battay, *Zeit. Unters. Lebensm.*, 1931, **61**, 161.
[164] A. Miermeister and F. Battay, *Deut. Weinblatt*, 1929, **3**, No. 10.
[165] H. Lundén, S. Reuterskiöld and S. Stare, *Svensk. Sockerfabr. Forenings*, 1927, Mar. 22nd, p. 3.
[166] G. Mezzadroli and E. Vareton, *Riv. Ital. Essenze Profumi*, 1931, **13**, 331.
[167] H. Mohler and H. Benz, *Mitt. Lebensm. Hyg.*, 1934, **25**, 47.
[168] J. Bruckner, *Biochem. Zeit.*, 1934, **270**, 346.
[169] J. W. Woodrow and A. R. Schmidt, *Phys. Rev.*, 1933, **43**, 379.
[170] E. Baier and H. Barsch, *Zeit. Unters. Lebensm.*, 1929, **57**, 224.
[171] G. Büttner and A. Miermeister, *ibid.*, 1929, **57**, 431.
[172] J. Tillmans, H. Riffart and A. Kühn, *ibid.*, 1930, **60**, 61.
[173] R. Pannier, *Ann. Falsif.*, 1934, **27**, 293.
[174] M. Haitinger, Jörg. and V. Reich, *Zeit. angew. Chem.*, 1928, **21**, 815.
[175] C. von der Heide, *Wein. u. Rebe*, 1929, **11**, 251.
[176] K. Elbe, H. Pfeiffer and A. Bretschneider, *Zeit. unters. Lebensm.*, 1933, **65**, 100.
[177] K. Braunsdorf and H. Brinckmeier, *ibid.*, 1933, **66**, 301.
[178] K. Braunsdorf, *ibid.*, 1934, **67**, 451.
[179] H. Mohler and J. Hartnagel, *Mitt. Lebensm. Hyg.*, 1934, **25**, 265.

[180] J. E. Heesterman, *Zeit. Unters. Lebensm.*, 1933, **66**, 309.

[181] J. Khouri, *Bull. Acad. Méd.*, 1932, **107**, 1027; through *Brit. Chem. Abst.*, *B*, 1934, 38.

[182] S. Pickholz, *Brauw. Malzind.*, 1932, **25**, 20; *Zeit. Ges. Brauw.*, 1933, **56**, 36.

[183] A. Miermeister and F. Battay, *Zeit. Unters. Lebensm.*, 1931, **61**, 161; *Deut. Weinblatt*, 1929, **3**, 10.

[184] G. Klein and H. Linser, *Osterr. Bot. Zeit.*, 1930, **79**, 125.

[185] C. Dhéré, Abderhalden's "Handbuch der Biologischen Arbeitsmethoden," II, 3, 4 (Berlin: Urban and Schwarzenberg, 1933).

[186] E. Abderhalden, *Biochem. Handlex.*, 1911, **2**, 313, 360.

[187] B. Lustig and G. Botstiber, *Biochem. Zeit.*, 1928, **202**, 84.

[188] A. Niethammer, *Zeit. Unters. Lebensm.*, 1929, **57**, 354.

[189] Schulze, *Zeit. Physiol. Chem.*, 1890, **14**, 522.

[190] O. Rosenheim, *Biochem. Jour.*, 1916, **10**, 176.

[191] Meesemaecker, *Compt. rend.*, 1930, **190**, 216.

[192] O. Rosenheim and R. K. Callow, *Biochem. Jour.*, 1931, **25**, 74.

[193] G. Lunde, E. Mathiesson and E. Mikkelsen, *Tidsi. Hermetikind.*, 1933, **19**, 375; Dept. Comm., Washington, *Tech. News. Bull. Bur. Standards*, 1927, No. 127.

[194] J. Becker, *Munch. Med. Woch.*, 1926, **73**, 99.

[195] D. Cortese, *Ind. Chimica.*, 1934, **9**, 1048.

[196] J. Grant, *Nature*, 1934, **134**, 365.

[197] J. Grant and H. Procter-Smith, *Analyst*, 1934, **59**, 749.

[198] A. R. Tankard, *ibid.*, 1934, **59**, 819.

[199] J. Grosbüsch, *Zeit. Anal. Chem.*, 1934, **99**, 12.

[200] A. Karsten, *Zeit. Getreide Mühlen- u. Bäckereiwesen*, 1935, **22**, 33.

[201] A. Castiglioni, *Ann. Falsif.*, 1935, **28**, 24.

[202] F. H. Cohen, *Acta Brev. Neerland.*, 1934, **4**, 46.

[203] B. Josephs, *ibid.*, 1934, **4**, 46.

[204] R. A. Peters, *Nature*, 1935, **135**, 107.

[205] L. Genevois, *Bull. Soc. Chim.*, 1934, (i), 1504.

[206] L. Genevois and L'Espil, *ibid.*, 1934, (i), 1498, 1504.

[207] L. E. Boober, *Jour. Biol. Chem.*, 1935, **107**, 591.

[208] F. Feigl, *Mikrochem.*, 1935, **17**, 29.

[209] J. Guillot, *Ann. Falsif.*, 1935, **28**, 75; Lunde, Kringstad and Weedon, *Zeit. Angew. Chem.*, 1933, **46**, 796.

[210] J. Guillot, *Ann. Falsif.*, 1935, **28**, 69.

[211] W. Diemair, B. Bleyer and F. Arnold, *Zeit. Unters. Lebensm.*, 1934, **68**, 364, 457.

[212] L. B. Pett, *Biochem. Jour.*, 1935, **29**, 937.

[213] J. Grant, *Brewing Trade Rev.*, 1935, July; *Brewers' Digest*, 1938, **13**, 41.

[214] W. Ciusa, *Olii e grassi*, 1935, **15**, 149.

[215] R. G. Harry, *Analyst*, 1936, **61**, 403.

[216] R. H. Common, *ibid.*, 1937, **62**, 284.

[217] A. de Clercq, *Natuurw. Tijdschr.*, 1937, **19**, 27.

[218] H. W. Kennersley, J. R. O'Brien and R. A. Peters, *Biochem. J.*, 1935, **29**, 2369.

[219] O. Warburg and W. Christian, *Ber.*, 1936, **69B**, 228.

[220] G. C. Supplee, S. Ansbacher and Bender, *J. Biol. Chem.*, 1935, **110**, 365.

[221] C. H. Whitnah, L. Kunerth and M. M. Kramer, *J. Amer. Chem. Soc.*, 1937, **59**, 1153.

[222] S. M. Weisberg and I. Levin, *Ind. Eng. Chem. Anal.*, 1937, **9**, 523.

[223] F. H. Cohen, *Arch. néerland. Physiol.*, 1935, **20**, 167.
[224] F. H. Cohen, *Rec. Trav. chim. Pays-Bas*, 1935, **54**, 133.
[225] G. C. Supplee, S. Ansbacher, G. E. Flannigan and Z. M. Hanford, *J. Dairy Sci.*, 1936, **19**, 215.
[226] G. N. Murthy, *Indian J. Med. Res.*, 1937, **24**, 1083.
[227] G. C. Supplee, G. E. Flannigan, Z. M. Hanford and S. Ansbacher, *J. Biol. Chem.*, 1936, **113**, 787.
[228] G. Hermann, *Zeit. Fleisch- u. Milchhyg.*, 1937, **47**, 258.
[229] S. Kaloyéréas, *Compt. rend. Congr. int. tech. chim. ind. agr.*, 1937, **2**, 346.
[230] M. A. Pyke, *Biochem. J.*, 1937, **31**, 1938.
[231] B. C. P. Jansen, *Rec. Trav. chim. Pays-Bas*, 1936, **55**, 1046; *Chem. Weekblad*, 1938, **35**, 565.
[232] R. C. Wright and E. B. Anderson, *Analyst*, 1938, **63**, 252.
[233] K. M. Henry, J. Houston and S. K. Kon, *Chem. and Ind.*, 1938, **57**, 974.
[234] J. Straub, G. A. Van Stijeren and W. J. Kabos, *Chem. Weekblad*, 1937, **34**, 732.
[235] J. Straub and W. J. Kabos, *ibid.*, 1938, **35**, 739.
[236] A. Steinmann, *Zeit. Unters. Lebensm.*, 1936, **71**, 358, 446.
[237] G. D. Dejardin and L. Herman, *Compt. rend.*, 1936, **202**, 651.
[238] C. F. Van Oyen, " Cold Storage," Mar. 19, 1936.
[239] H. Barsch, *Z. Anal. Chem.*, 1935, **101**, 388.
[240] R. Cantieri, *Helv. Chim. Acta*, 1936, **19**, 96.
[241] H. J. Vlezenbeek, *Pharm. Weekblad*, 1937, **74**, 127.
[242] M. Déribéré, *Bull. Assoc. Chim.*, 1938, **55**, No. 2.
[243] A. Amati and G. Bolzano, *Chimica*, 1931, **7**, 152.
[244] A. R. Tankard, *Analyst*, 1938, **63**, 815.
[245] G. Barger, F. Bergel and A. R. Todd, *Nature*, 1935, **136**, 259.
[246] R. A. Peters, *Nature*, 1935, **135**, 107.
[247] R. T. Wagner Jauregg, F. W. Klaveren and H. Vetter, *Zeit. Physiol. Chem.*, 1935, **234**, 196.
[248] G. Barger, B. C. P. Jansen and A. R. Todd, *Chem. and Ind.*, 1935, **54**, 596.
[249] J. Goudsmit and H. G. K. Westenbrinck, *Nature*, 1937, **139**, 1108.
[250] H. G. K. Westenbrinck and J. Goudsmit, *Rec. Trav. chim. Pays-Bas*, 1937, **56**, 803.
[251] W. Karrer, *Helv. Chim. Acta*, 1937, **20**, 1147.
[252] M. A. Pyke, *Biochem. J.*, 1934, **28**, 602.
[253] M. A. Pyke, *Nature*, 1938, **141**, 1141.
[254] J. R. Nicholls, *Analyst*, 1929, **54**, 335.
[255] M. Déribéré, *Bull. Assoc. Chim.*, 1938, **55**, No. 2.
[256] Anon, *Analyst*, 1935, **60**, 615.
[257] S. J. Lewis, *Nature*, 1939, **143**, 316.
[258] J. A. Wiegand, *Arch. néerland. Physiol.*, 1938, **23**, 281, 312, 331.
[259] D. J. Hennessy, and L. R. Cerecedo, *J. Amer. Chem. Soc.*, 1939, **61**, 179.
[260] H. G. K. Westenbrinck and B. C. P. Jansen, *Acta Brev. Neerl. Physiol.*, 1938, **8**, 119.
[261] J. Houston and S. K. Kon, *Nature*, 1939, **143**, 558.
[262] H. F. Pedersen, *Dansk. Tids. Farm.*, 1938, **12**, 137.

ADDITIONAL REFERENCES.

R. Nesini, *Prag. Arch. f. Tiermed.*, 1933, **12**, 237 (General article on foods).

J. L. Deuble and R. E. Schoetzow, *J. Amer. Pharm. Ass.*, 1931, **20**, 655 (Olive oils).

A. Niethammer, *Biochem. Zeit.*, 1929, **209**, 447 (Qualitative determination of fats by micro methods).

G. B. Mafei, *Atti Inst. Botan. Universita di Pavia*, 1932, Vol. 3, Feb. (Differentiation of poisonous from edible fungi).

J. M. Rieck, *Gordian Zeit. f. Kakaogewinnung usw.*, 1929, **35**, 827, 828 (Cocoa butter).

H. Fincke, "Die Kakaoprüfung und ihre Verfälschung," Abschnitt 1 Stuttgart, 1929 (Testing cocoa butter).

K. Eble, H. Pfeiffer and R. Bretschneider, *Zeit. Unters. Lebensm.*, 1933, **65**, 100 (Detection of removal of "Mark of Origin" from eggs).

C. von der Heide, *Wein. u. Rebe*, 1929, **11**, 251 (Criticism of capillary method for grape wine detection).

G. Mezzadroli and E. Vareton, *Ind. suc. ital.*, 1929, **22**, 3 (Sugar manufacture).

A. Baud and Courtois, *Jour. Pharm. Chim.*, 1928, **120**, 215 (Olive oils).

A. Baud and Courtois, *Chemist and Druggist*, 1924, **110**, 325 (Olive oils).

A. Baud, *Pharm. Acta Helvet.*, 1933, **8**, 83 (Olive oils).

A. Lender, *ibid.*, 1933, **8**, 83 (Olive oils).

E. J. Parry, *Chemist and Druggist*, 1925, **111**, 92 (Olive oils).

H. Marcelet, *Chim. e. Ind.*, 1929, **21**, 527 (Testing marine oils).

P. R. Peacock and S. Wright, *Jour. Physiol.*, 1926, **61**, 36 (Cod liver oil).

R. Nesini, *Tierarzt. Rundschau*, 1933, **39**, 266 (Foodstuffs in general).

J. Muir, *Food*, 1933, **3**, 95 (General).

G. Wundram and F. Schönberg, *Berl. Tierärztl. Woch.*, 1934, **50**, 107 (Examination of hen's eggs).

K. Weber, *Chem.-Ztg.*, 1932, **56**, 642 (Estimation of sodium nitrite in preserving salt).

J. Khouri, *Bull. Acad. Méd.*, 1932, **107**, 1027 (Grape wines).

J. Khouri, *Ann. Falsif.*, 1932, **25**, 583 (Wines).

J. Grossfeld, *Zeit. Unters. Lebensm.*, 1931, **62**, 441 (Cocoa butter).

J. Grant, *Chem. and Ind.*, 1934, **53**, 1074 (General).

Lustig and Botstiber, *Biochem. Zeit.*, 1928, **202**, 81 (Identification of fats and mixtures).

B. Zimmermann, *Zeit. Zuckerind. Czechoslav.*, 1930, **54**, 405 (Sugar refineries).

E. Naehring, *Deut. Zuckerind.*, 1932, **57**, 1007 (Sugar industry).

A. G. Nasini, *Ind. Saponiera*, 1929, **24**, 18 (Olive oils).

R. Kuhn, P. György and T. Wagner-Jauregg, *Ber.*, 1933, **66**, 1034 (Lactoflavin and the fluorescence of milk).

E. Gründsteidl, *Zeit. ges. Mühlenwesen*, 1931, **18**, 224 (Fluorescence microscopy of flours).

E. Gründsteidl, *Mühle*, 1930, **67**, 921 (Bakery).

K. Braunsdorf and W. Reidemeister, *Zeit. Unters. Lebensm.*, 1934, **68**, 59 (Age of eggs).

A. S. Karsten, *Milchwirtsch. Zentr.*, 1934, **63**, 225.

R. Baetslé and J. De Wever, *Ann. Soc. Brass.*, 1934, July-Aug. (Fluorescence analysis in the brewery).

J. Voss, *Angew. Bot.*, 1934, **16**, 510 (Wheat).

W. Diemair, B. Bleyer and F. Arnold, *Zeit. Unters. Lebensm.*, 1934, **68**, 457 (Wines).

F. Provvedi, *Olii e grasse.*, 1934, **14**, 121 (Considers lamp of doubtful value for examining olive oils).

A. S. Karsten, *Zeit. Fleisch- u. Milchhyg.*, 1935, **45**, 301 (Milk and meat. General).

H. and A. E. Rolle, *Compt. rend.*, 1935, **201**, 1434 (Irradiated cholesterol emits rays of wavelength 3533 and 3572 Å.).

E. R. Bolton and K. A. Williams, *Analyst*, 1938, **63**, 84 (Fuel oil in whale oil).

H. Marcelet, *Jour. Pharm. Chim.*, 1936, **24**, 213 (Effects of refining processes on the fluorescence of olive and arachis oils).

R. Müller, *Klin. Woch.*, 1937, **16**, 807 (Effects of diet on the fluorescence of human milk).

M. Déribéré, *Bull. Assoc. Chim.*, 1939, **56**, No. 1 (Fluorescence of the flavins).

CHAPTER VII.

FUELS AND LUBRICANTS.

THE appearance of coals and oil-shales in ultra-violet light has been studied by H. Briggs.[1] In general, *coal* shows but little of interest, but in one specimen examined the bands of clarain had a golden speckled appearance, although the ash was without any characteristic fluorescence. *Ankerite*, which often occurs with coal, has a golden-yellow fluorescence.

The oil-shale group, however, gave interesting results. Common shales or "Blaes" appeared very dark brown in the massive state, although the colour was sometimes absent from the powdered form. With a kerogen shale, however, a rich chocolate-brown was observed in both the powdered and the lump forms. Torbanite and cannel can be differentiated readily by their fluorescence, since the former shows bright yellow streaks on a brown background.

M. Wittich,[2] who investigated Esthonian and Manchurian shale-oils, found that useful indications can be obtained as to their origin, preparation and process of cleaning, and that individual fractions can be graded according to their boiling-points from the fluorescence.

Bitumens, etc.[45]—Von Skopnik,[3] Macht[25] and Herrmann[26] all mention some uses of the lamp in certain road-construction laboratories for the identification of bitumens, and G. Testi and G. Mardi[12] have examined sections of bituminous stones by the method of impregnation. On the other hand, it does not appear possible to identify various bitumens in admixture with one another, even by examination of their solutions. This conclusion is confirmed by S. Bruckner and P. Meinhard[13] and by W. Körsten.[14]

The former workers also studied coal tar, petroleum and stearin pitches and crude montan wax, each of which gave a characteristic

fluorescence suitable for identification of the substance in the pure state, but not in admixture. It is advisable to work not only with the solid material, but also with solutions in chloroform, or benzene, and the latter may also be allowed to evaporate on filter-papers.

The ultra-violet absorption of hydrocarbon fuels noticed by Schon and Bendix-Nielsen [30] is probably due to *unsaturated constituents*, and is a measure of purity as indicated by the sulphuric acid colour test. Yellow fluorescent spots or patches due to anthracene are plainly visible in tarmacadam preparations containing this compound, and one of us (J. G.) has found that its presence may be confirmed by removing it by controlled sublimation and testing the sublimate. According to M. A. Iljinsky and B. I. Afremoff,[40] Ukranian anthracene may be distinguished from Siberian anthracene by the very weak fluorescence of the former.

Mr. H. B. Milner has been kind enough to send us the following details of the work on bitumens carried out under his direction at the Geochemical Laboratories, of which the following are extracts: "... it is possible to distinguish between many types of bitumens, both natural and artificial, with a rapidity unobtainable by the usual chemical and physical methods. ... The examination is best carried out by placing 5 c.c. of a 0·5 per cent. carbon disulphide solution of bitumen in a quartz tube and comparing the fluorescence with that given by a bitumen of known origin. Trinidad Lake asphalt, Cuban asphalt, Pyrimont Seyssel asphaltic limestone, Mexphalt *R*1 and Mexphalt *E* are employed as general standards (under the same working conditions). ... All the residual asphalts examined were characterised by a greenish-yellow fluorescence which was brighter in colour than the greenish-brown of blown asphalts. Asphaltic limestones were found to exhibit fluorescences somewhat yellower than those given by residual asphalts. ... When the colour and intensity of the meniscus is observed, ... differences may be detected in otherwise apparently identical solutions. When the concentration of a solution is increased beyond 0·5 per cent., the distinction between different bitumens becomes less marked while the fluorescence tends to assume a dirty brownish colour. ... Further, with unknown materials containing mineral matter, it becomes necessary to determine the bitumen content in order

to prepare a solution of the requisite concentration. Filtration is not, however, necessary, since the presence of the mineral filler has no effect on the fluorescence in the neighbourhood of the meniscus, provided the solution is allowed to stand for a few minutes to allow the filler to settle. At present, the disadvantage of the method is that it has not been found possible to keep the standards without deterioration, even in the dark. Experiments are at present in progress to ascertain whether they can be preserved by excluding both light and oxygen.

"An alternative method of examination is to place one drop of filtered 0·5 per cent. solution of bitumen on a filter-paper, and note the changes which take place on evaporation of the solvent. The yellowish fluorescence initially seen with asphaltic bitumen, changes to a brown spot surrounded by a yellowish-white rim, and by comparison with known materials, it is sometimes possible to determine the character of the bitumen without further examination. The fluorescence rapidly changes, however, and it is difficult to prepare satisfactory standards. . . ." Milner's experiences with road-making materials of the bitumen type are cited on p. 112.

If, however, the solutions of bitumens are used, the fluorescence colours may be matched against those of calibrated standards consisting of solutions of appropriate dyestuffs in alcohol or benzene (I. Sala [38]). It should be noted that the fluorescence of bitumen is dependent to some extent on temperature, and that this dependence is reversible. Sala also recommends the method for the differentiation of rock asphalts and petroleum products. G. Hradil [43] has applied the methods of fluorescence spectroscopy to bitumen research.

W. Teuscher [4, 5] states that 1 part in 50,000 of *coal-tar pitch*, which has a green-blue fluorescence, is detectable in asphalt (petroleum pitch) which fluoresces with a redder shade. The method is not suitable, however, for the detection of asphalt in the pitch. Solutions of pitch or bitumen, and mixtures of the two in an inert solvent such as benzene or acetone, can also be differentiated in ultra-violet light. W. Becker [6] has elaborated a method for the estimation of the components of mixtures of tar and bitumen which is rapid and has an accuracy of 5 per cent. The substances are dissolved in petroleum spirit, and the colours are compared under the lamp with those from the standard

mixtures of known compositions. The fluorescence phenomena of benzene, paraffin and similar *liquid fuels* are also dealt with on pages 295 *et seq.*, but it may be mentioned here that paraffin oil, which normally does not fluoresce, appears blue if more than 5 per cent. of petroleum (spirit or jelly) is present.[9] According to A. Bentz [27] crude oils (of German origin) have a yellow or brown fluorescence when spotted on filter-papers, whilst refined oils develop a light blue colour. Light oils containing benzene also appear yellow, but when the benzene (and asphaltenes) are removed this turns progressively brown, and eventually all fluorescence vanishes.

Lubricants, etc.—J. Tausz and A. Rabl [7] state that sulphur dioxide suppresses the fluorescence of *mineral oils*, but that on exposure to air this gas is lost and the fluorescence returns.[11, 42] The amount required to produce this effect was found to vary from 0·3 mgrm. of SO_2 per c.c. for Russian oils to 9·4 mgrms. for some American oils. K. Trautzl [15] has found (after Brooks and Bacon [31]) that aromatic nitro-compounds also remove the fluorescence of mineral oils, and C. H. Liberalli [16] has described a method for the detection of mineral oils in turpentine; investigations have also been carried out on transformer oils, particularly from the point of view of checking deliveries against standards.

Lubricating oils and greases have been dealt with by J. Muir [10] who recommends examination of the oil in a thin film on a glass slide, or impregnated into filter-paper. Blue to violet colours were usually obtained, but the most interesting observation is the fact that oils which tend to "gum" on exposure develop simultaneously a green or yellow fluorescence. Motor grease, ordinary lubricating grease and railway waggon grease appeared violet, lavender and pink, respectively. M. Freund [29] mentions an interesting *accelerated gumming test* in which the fuel (*e.g.*, gasoline) is exposed for 10 minutes to a mercury arc, and the gum then determined in the usual way. The results for potential gum content agree closely with storage tests, and the gums produced in both cases have similar carbon/hydrogen ratios. Routine tests for mineral oil in brake fluids have also been devised.

In a similar way A. Balada [36] has been able to follow the oxidation of *insulating oils* and the formation of asphalt in motor lubricating oils (see also Radley [44]); asphalt in cylinder oils was

detectable, and the amount could be estimated by making comparisons in ultra-violet light against standards of known composition. F. Evers [37] however, prefers the Zeiss photometer for the evaluation of the fluorescence of oils, and uses a cadmium sulphide glass as standard. Balada also found that in general, naphthenic oils, asphaltic oils and oils derived from a paraffin base had a blue, brown and pale yellow fluorescence, respectively, these differences being more marked in the fractions of high viscosity.

The peculiar " bloom " of a mineral oil, which is associated with its powers of fluorescence, is regarded by some as an indication of quality, and a number of patents protect the addition of substances for this purpose. Examples [32-35, 41] are the residuum from cracking processes ; [17] extracts of coal-tar pitch ; [18-20] a mixture of naphthenic acid and acridine orange base (added to the hot oil) ; [21] amino-2-phenyl benzthiazole or a similar oxazole or perylene derivative ; [22, 33] and asphaltenes from polymerised hydrocarbon oils.[23, 24]

Finally a word of caution in connection with the above materials (and, indeed, with many others discussed in this book) arises from the fact that the fluorescence of many of them changes or disappears after exposure, sometimes only for a short while, under the lamp (see especially Evers [28] on transformer- and switch-oils). The nature of the fluorescence should therefore be noted as rapidly as possible and the sample removed or covered.

Other papers on fuels and the *petroleum industry* have been published by F. Dangl,[8] but are of general interest only, but reference should also be made to p. 224 where the examination of such products for Customs purposes is described, and to p. 369 where the examination of oil stains on textiles and the control of quality of oils used in textile work are discussed. A description [39] of an up-to-date laboratory for the testing of petroleum products makes reference to provision for fluorescence analysis.

REFERENCES.

[1] H. Briggs, *Trans. Inst. Mining Eng.*, 1929, 77, 1, 11.
[2] M. Wittich, *Brennstoff-Chem.*, 1927, No. 19.
[3] Von Skopnik, *Chem. Ztg.*, 1928, 52, 397.
[4] W. Teuscher, *ibid.*, 1930, 54, 987.
[5] W. Teuscher, *Chem. Fabrik*, 1930, 53.

[6] W. Becker, *Asphalt und Teer*, 1930, 30, 87.
[7] J. Tausz and A. Rabl, *Erdöl u. Teer*, 1931, 7, 106.
[8] Dangl, *Petroleum*, 1931, 27, 2.
[9] C. H. Liberalli, *Rev. Soc. Brasil. Chim.*, 1932 (through *Brit. Chem. Abst.*, *B.*, 1932, 1040).
[10] J. Muir, *Ind. Chem.*, 1932, 8, 437.
[11] F. Evers and R. Schmidt, *Petroleum*, 1932, 29, 1.
[12] G. Testi and G. Mardi, *Ann. Chim. Applic.*, 1927, 17, 446.
[13] S. Bruckner and P. Meinhard, *Petroleum*, 1933, 29, 8.
[14] W. Körsten, *Teer u. Bitumen*, 1932, 30, 417.
[15] K. Trautzl, *Erdöl u. Teer*, 1931, 7, 193.
[16] C. H. Liberalli, *Rev. Soc. Brasil. Chim.*, 1932, 3, 19; *Boll. Ass. Brasil. Pharm.*, 1932, 13, 363.
[17] Hanna, Cushman and Doell, Standard Oil Co., California, U.S.P. 1,566,000, 15/12/25.
[18] Black, Rial and McConnell, Pan Amer. Pet. Co., U.S.P. 1,708,563, 9/4/29; 1,708,602, 9/4/29.
[19] Black, Rial and McConnell, Richfield Oil Co., U.S.P. 1,839,012, 29/12/31; 1,842,856, 26/1/32.
[20] Rial and Barrett, Pan Amer. Pet. Co., U.S.P. 1,708,593, 9/4/29.
[21] Cassidy, U.S.P. 1,860,850, 31/5/32.
[22] I.G. Farbenind., B.P. 390,188, 27/3/33; Fr. P. 767,250, 12/7/34.
[23] Smith, Gulf Refining Co., U.S.P. 1,868,473, 7/9/32.
[24] Miller, Standard Oil Co., Indiana, U.S.P. 1,869,608, 8/2/32.
[25] F. Macht, *Erdöl u. Teer*, 1931, 7, 225.
[26] Herrmann, *Asphalt u. Teer Strassenbautech.*, 1930, 30, 9.
[27] A. Bentz, *Jahrb. Deut. Nat. Komitees f. d. Internat. Bohrkongresse*, 1932, 2, 17.
[28] F. Evers, *Zeit. angew. Chem.*, 1931, 44, 323.
[29] M. Freund, *Proc. World Pet. Cong.*, 1933, 2, 108.
[30] S. A. Schon and I. Bendix-Nielsen, *Arch. Pharm.*, 1934, 272, 761.
[31] B. T. Brooks and R. F. Bacon, *Jour. Ind. Eng. Chem.*, 1914, 6, 623.
[32] Standard Oil Co., E.P. 758,155.
[33] J. W. Orelup, E.P. 424,205; U.S.P. 1,987,309, 8/1/35.
[34] S. Landa and J. Cech, *Coll. Czech. Chem. Comm.*, 1934, 6, 423.
[35] A. Homer and J. E. Purvis, *Jour. Amer. Chem. Soc.*, 1907, 91, 1103; 1908, 93, 1319.
[36] A. Balada, *Petroleum*, 1935, 31, 11.
[37] F. Evers, *Erdöl u. Teer*, 1936, 12, 457.
[38] I. Sala, *Strade*, 1938, 20, 197; *Road Abst.*, 1938, 5, No. 228.
[39] Anon, *Ind. Chem.*, 1936, 12, 409.
[40] M. A. Iljinsky and B. I. Afremoff, *Ber.*, 1936, 69, 1824.
[41] J. A. Radley, *Chem. Age*, 1935, 32, 480.
[42] S. V. Grechishkin and M. V. Kurbin, *J. Tech. Phys. U.S.S.R.*, through *Amer. Chem. Abst.*, 1937, 31, 247.
[43] G. Hradil, *Petroleum*, 1934, 30, 1.
[44] J. A. Radley, *Elec. Times*, 1936, Feb. 13.
[45] Anon., *Strade*, 1938, 20, 197; through *Road Abst.*, 1938, 5, 228.

CHAPTER VIII.

INORGANIC CHEMISTRY.

INORGANIC substances in the pure state usually show little or no fluorescence in ultra-violet light.[28, 114] This was first observed by H. Lehmann,[1] and we shall see that a number of organic substances which have been regarded until recently as examples of fluorescence "par excellence" behave in a similar manner. Chemicals pure enough for technical purposes often have a strong fluorescence, and a number of examples of the influence of impurities (*e.g.*, in zinc, mercury and boron compounds) are cited below. Radioactive elements are dealt with on pp. 201 and 269.

Alkali Metals.—E. Becquerel (1867) first noted[35] that the hydroxides of these metals fluoresce when ordinary commercial impurities are present, and A. Grumbach and M. Ribaillier[36] attribute the colours of freshly-cut potassium hydroxide (green) and sodium hydroxide (blue) to the presence of formates; the increase in brilliance which occurs on heating to the fusion-point is followed by a change in colour to yellow (due probably to carbonates) and then a disappearance of the fluorescence. This theory is confirmed by the fact that pure formates have no fluorescence, but if subjected to heat in such a way that carbonates are formed a yellow fluorescence develops; otherwise it turns blue in colour. S. Oxa and S. Yagi[37] found that halides of the alkali metals prepared by crystallisation from solutions containing manganese, tin and lead ions are strongly luminescent in light of wave-lengths 2300 to 3600 and 3100 to 3900 Å.; this phenomenon is associated with deformation of the crystal lattice by the heavy metals, and with an increased degree of transparency. Salts of the alkali metals can also act as inhibitors or activators of fluorescence (see Uranium and p. 198).

O. Wolf[2] noted that commercial calcined potassium carbonate gave a reddish luminescence which was, however, confined to small patches; he showed that this was due to the presence of

potassium sulphide in small traces and that the pure salt does not fluoresce.

Mercury (see also Drugs, p. 117 and Martin [33]). An interesting experiment is based on the fact that the fluorescence of mercuric chloride is due to the presence of mercurous chloride. A sheet of paper covered with a layer of finely-powdered mercuric chloride, which must be perfectly pure, is placed under the lamp. A globule of mercury is now rolled across the powder and leaves a fluorescent path, due to the formation of traces of mercurous chloride by the contact of the mercury with mercuric salt. I. Plotnikov [34] has shown, however, that mercurous chloride is obtainable in the non-fluorescent state (*e.g.*, when it separates from Eder's solution), although on adding commercial calomel to it a bright orange colour develops. L. R. Biggs [98] uses the fact that mercury vapour is permeable to ultra-violet light as the basis of a test for this element.

Bromine.—Since the action of nascent bromine on fluorescein at *p*H 5·5 is to convert it into eosin, the change in the colour of the fluorescence may be used as a qualitative test for 10^{-6} grm. of bromides even in the presence of chlorides (Ganassini [51] and Hahn [40]). The reaction is best carried out with a piece of filter-paper which is impregnated with a solution of fluorescein and dried, the bromide being mixed with acetic acid and lead dioxide, and heated in a test-tube, the neck of which is drawn out to a capillary of diameter 2 mm., and placed in contact with the filter-paper. Similarly the yellow fluorescence of resorufin in an alkali carbonate solution is destroyed by the action of chlorine or bromine.[66, 67]

A similar but improved technique is described by W. M. Seaber [79] who evolved an apparatus of the type used in the Marsh-Berzelius test for arsenic, the bromine being liberated by means of a 20 per cent. solution of ammonium persulphate and passed through a filter-paper which had been soaked in a solution of fluorescein in alcohol. Quantitative results were obtainable by matching the resulting red spots against those produced in a similar way from standards of known bromide content. The method was evolved principally as a test for damage by sea-water (which contains small quantities of bromides), and in ordinary light a stain corresponding with 0·007 mgrm. of bromine was visible. J. Grant [80] subsequently showed that if the stains were

examined in ultra-violet light, not only was the sensitiveness raised (the stain from 0·0017 mgrm. of bromine being easily visible), but that the ease of matching was greater, the eosin stain appearing as a brown spot against a vivid golden background. The presence of carbon (from dirty air) heightened the contrast. A similar quantitative method which is also applicable to bromates, is described by F. L. Hahn,[81, 84] although it has not been used in ultra-violet light.

Although it does not come strictly within the domain of fluorescence analysis, the observation of H. Demassieux and V. Henri[15] on the *determination of bromine in hydrochloric acid* is of interest, since most chemical methods fail for less than 1.0 mgrm. per litre. Comparison of the ultra-violet absorption spectrum with that of pure hydrochloric acid enables 0.1 mgrm. per litre of bromine to be detected. The effect of bromine on the absorption spectrum of 10 N hydrochloric acid is 10^3 times as great as the effect of the same amount of bromine on that of water. "Pure" hydrochloric acid was found by such methods often to contain traces of bromine. Probably a compound of bromine and hydrochloric acid is formed whose ultra-violet absorption (at about 2100-2500 Å.) is greater than that of bromine itself.

The use of fluorescent indicators for precipitation titrations of the silver-halide and zinc-ferrocyanide type are dealt with on page 316, by H. R. Fleck, R. F. G. Holness and A. M. Ward,[65] and by J. Grant.[82] The halogen ions frequently act as inhibitors of fluorescence[76] (*cf.* p. 198).

Haitinger, Feigl and Simon[29] have examined the fluorescence colours of over 200 compounds, and their paper contains the results in tabular form. The majority of the compounds examined gave a red-violet fluorescence, so that this table is of little use for identification; 62, however, gave definite fluorescence colours. It is interesting to notice that the low-valency compounds have a more characteristic fluorescence than high-valency compounds, and that except in the case of uranium compounds, only a few coloured compounds fluoresce (see Table 12, p. 207).

An interesting method for the detection of *phosphorus* utilises the phosphorescent glow of this element. It is due to R. Gros[83] who distils the sample containing white phosphorus in the dark under a pressure of 15 mm., and observes the resulting glow.

The sensitiveness is 0·001 mgrm. in 200 grms. of sample. According to H. Herrmann [123] 10 μ-grm. of phosphoric acid produces a brown-green and green fluorescence with fuchsin and victoria blue, respectively.

With the aid of the fluorescence microscope (see p. 78), W. Lenz [3] has investigated the fluorescence of a number of inorganic salts (Table 12). He did not, however, use a filter to eliminate visible light, and the use of such a filter would probably have modified to some extent the fluorescence colours recorded for some of the compounds. He examined some highly-coloured compounds, among which were mercury and silver iodides, cobalt and mercury thiocyanates, nickel dicyanodiamine, and ammonium and potassium platino-cyanides, some of which gave a strong characteristic fluorescence much more apparent in thin than in thick layers. In a general test for metals described by L. Szebellédy and H. Gaál,[52, 62] a solution of the salt is mixed with boric acid and cochineal at *p*H 5·8 to 6·9 (phosphate buffer), when characteristic fluorescence colours are obtained; of the anions only carbonates interfere. This may also be used as a test for boron (*infra*).

Of the *arsenic* compounds the trioxide and the tri-iodide show a strong bluish-white, and a light yellowish-white fluorescence, respectively, whilst calcium and magnesium ammonium arsenates have a whitish shimmer. A. A. King [4] has introduced a delicate test for arsenic using the lamp. If the mercuric chloride paper used in the Gutzeit test is placed under the lamp, then the presence of small traces of arsenic, insufficient to produce a discoloration visible in ordinary light, is plainly seen as an almost black appearance of the paper. The test is claimed to detect the presence of 0·000,001 grm. of arsenic, and might be of use in biological or toxicological work.

Lead has been titrated with sodium carbonate with sodium fluoresceinate as indicator (*cf.* S. N. Roy [102]), and as with similar reactions involving the use of this indicator, it can with advantage be carried out in ultra-violet light (*cf.* Chapter XIV).

Another delicate test is for *uranium compounds*,[105, 107] and has been introduced by J. Papish and L. E. Hoag,[5] who applied the observation made by E. Nichols and M. K. Slatterly,[6] that traces of uranium salts fused with sodium or potassium metaphosphate, borax, or with sodium or calcium fluoride, give a bead which

has a strong lemon-yellow fluorescence. Papish and Hoag used sodium fluoride, but with this salt the test is not completely specific as niobium gives a similar fluorescence. With potassium fluoride, however, a fluorescent bead is obtained, the luminescence of which is weaker, although the reaction is characteristic of uranium alone. In cases of uranium poisoning, the kidney cortex is the only organ in which the uranium is deposited, and H. Eitel [7] recommends that the ash from the organ should be fused with borax, and the bead so obtained examined under the lamp, when 1 part in 500,000 is detectable. According to F. Hernegger and B. Karlik [104] 10^{-10} grm. of uranium may be detected by the sodium fluoride method; and an application of the method to the determination of uranium in sea-water is also described.[122]

The measurement of the duration of fluorescence of uranium salts has been carried out by F. Perrin,[8] and it should be mentioned here that Achard and his co-workers [45] have extended Perrin's work, and have demonstrated the inhibition of this fluorescence by compounds of an antioxygenic nature (*vide supra*). Alkaloids, aniline, orcinol, tannic acid, phenol, cresol and the ions S′, CNS′, I′, NO'_2, $S_2O''_3$, $S_2O''_4$, $HAsO''_3$ were inhibitors, whilst sugars, urea, alcohols, pyridine, acetamide and the ions NO'_3, Cl′, Br′, F′, CN′, CO''_3, SO''_4, AsO'''_4, PO'''_4 and AcO′ were inactive. For low concentrations of the above salts of the alkali metals, the intensity of the fluorescence was found to be a decreasing exponential function of the concentration of the metal or of the uranium (see p. 383). It is interesting to compare these results with those of Volmar, obtained in the case of quinine (see p. 314).

Small quantities of fluorescein heated with *boric acid* give a vividly-fluorescent substance, and N. F. Shirov [9, 73] has estimated the optimum concentration of the fluorescein (which is the activator) as 1 in 10,000. Below this concentration the spectrum lines shift towards the violet end, and above it a shift towards the red is noted. This reaction can be used as a test for boric acid, and responds to so small a quantity as 0·02 mgrm. (see p. 166). Another test due to L. Szebellédy and J. Gaál,[52, 62] makes use of the bright yellow-orange fluorescence of boric acid in the presence of a 0·5 per cent. tincture of cochineal at *p*H 5·8 to 6·9 (phosphate buffer). Aluminium and magnesium ions alter the shade of the fluorescence (although they produce no colour alone), and

lead, copper, cadmium, cobalt, nickel, iron, manganese and chromium impede its rate of appearance, but the reaction is otherwise specific (see above). The sensitiveness is normally 12 γ in 0·5 c.c. of solution, using 0·1 c.c. of reagent and 10 mgrms. of sodium acetate (to produce a *p*H value of 5·8 to 6·9); as a micro reaction the sensitiveness is 0·5 γ in 0·025 c.c. A yellow-green fluorescence is also obtained after heating boric acid with 0·1 per cent. of sucrose at 800° C.[69] L. Szebellédy and S. Tanay [89] found subsequently that thiosulphates, bromides, nitrates, cobalt or chromium, which interfere with the test for boric acid if this is carried out in daylight, are without effect if the observations are made in ultra-violet light. Iodides, however, must be eliminated by means of silver sulphate; chlorates must be reduced with formaldehyde; and antimony compounds should be oxidised by chlorine. Boric acid (*cf.* aluminium, p. 205) may be activated by means of certain dyestuffs and is then phosphorescent.[90] L. Rosenthaler [97] records the production of a red fluorescence when rufianic acid is added to a small quantity of boric acid; this is a very sensitive test.

J. Donau [10] has devised a microchemical test for the detection of the presence of *bismuth* and *manganese* compounds in calcium salts, and a test for *sulphites* is discussed under Preservatives (p. 166). A test for *peroxides* is mentioned on page 203, and hydrogen peroxide produces chemiluminescence if added to a solution of 0·1 grm. of luminol and 2 mgrms. of hæmin in 100 c.c. of 1 per cent. sodium carbonate solution, $0{\cdot}012 \times 10^{-6}$ grm. being detectable (W. Langenbeck and U. Ruge [91]).

Hydrosulphites and certain other reducing agents convert resazurin into resorufin (*cf.* p. 293) in the presence of an alkali carbonate, and the fluorescence changes from brown, through colourless, to red.[66, 67] *Nitrites* (but not nitrates) give a red fluorescence on addition of a 0·1 per cent. solution of Magdala red.[70]

In an independent investigation R. Robl [11] has examined the fluorescence of a number of different inorganic compounds in the form of powder, or in bulk, but some of his observations are not completely in accordance with those of Lenz (*loc. cit.*). A number of substances were also examined at high temperatures, when it was found that the fluorescence disappeared, but reappeared on cooling. Examples are, the chloride, bromide and

sulphate of lead (see Table 12). Commercial potassium hydroxide, lithium hydroxide, arsenious acid, phosphorus pentoxide and aluminium oxide containing organic salts, all fluoresce. The colour of the phosphorescence is in most of these cases the same as that of the fluorescence.

Beryllium.—H. L. J. Zermatten [38] describes a test for this element (*e.g.*, in minerals) in which the sample is fused with sodium carbonate, the melt being dissolved in 5 *N* hydrochloric acid and tetrahydroxy flavonol (see also aluminium) and sodium hydroxide added; a yellow-green fluorescence results. S. Satoh [39] found that a nitride of beryllium with a blue fluorescence could be produced by passing ammonia for 4 hours over beryllium containing 10 per cent. of alumina at 1000° C. The only other fluorescent nitrides known are those of aluminium and boron and these require activation by silicon and carbon, respectively (*cf.* the alkaline earths). E. Tiede and F. Goldschmidt [13] have examined the fluorescence of beryllium sulphide and S. Satoh [103] deals with the phosphorescence of the nitride.

Alkaline Earths.[105]—E. Beutel and A. Kutzelnigg [14, 15] have studied quantitatively the fluorescence of calcium oxide, calcium hydroxide, calcium sulphate and calcium carbonate, and the corresponding compounds of strontium and barium, by means of a photometer with red, green and blue filters, and have tabulated the colours in their papers. The tungstates of calcium and barium also provide an example of activation by heavy metals (*cf.* beryllium), 0·01 grm.-mol. of lead (as tungstate) being particularly effective, although an excessive quantity decreases the fluorescence.[44]

Zinc compounds were also examined by Beutel and Kutzelnigg (see also p. 208), and those found to have no fluorescence are given on page 208. The oxide, hydroxide, sulphide, basic carbonate and double potassium cyanide (blue-violet) have, however, varying degrees of fluorescence, which depend to a large extent on the method of preparation, state and origin. The rate of decay of phosphorescence depends on the size of the particle.[63] Zinc cyanide, smithsonite and hydrozincite also show a luminescence, and a rather indefinite fluorescence is shown by colloidal solutions of the oxide, sulphide and cyanide. It seems that so far as *zinc oxide* is concerned the purest product obtainable has definitely a fluorescence; this has been established by E. Beutel and A. Kutzelnigg [48] for the needle-shaped crystals obtained in

the electric furnace, for products from which cadmium was removed by combustion, and for those prepared by the decomposition of zinc methyl with water. Absorption of carbon dioxide or water, mechanical treatment (*e.g.*, compression or pulverisation) and heat treatment decrease the intensity of the fluorescence, a minimum in the last case being obtained after 30 minutes at 600° C. in the electric furnace. Heat treatment also appears to play some part in determining the colour, since this is yellow-brown and orange for high- and low-temperature processes, respectively, and green if reducing gases are present; decomposition of the carbonate at 300° C. gives the brown colour, but the nitrate loses its fluorescence. R. E. Lutz [47] has used the green fluorescence produced by urobilin in the presence of zinc as a micro-test for the latter, but as G. Bertrand and L. de Saint-Rat [86, 93] point out, this reagent provides a sensitive and specific colour test for copper, and if much of this latter element is present, the fluorescence due to the zinc will be masked.

Zinc sulphide is said to be non-fluorescent when pure, but highly-purified specimens still show some fluorescence, and indeed it is very difficult to prepare a specimen which is free from zinc oxide [114]; in order to prepare the fluorescent variety (*e.g.*, for luminous paints [23, 24]), it is first necessary to isolate the sulphide in the pure state by precipitation with hydrogen sulphide, and then to add an activator [110] (*e.g.*, 0·01 per cent. of copper as copper sulphate, the mixture being heated at 1150° C.[20, 22]). Iron should be absent (see Table 12). N. F. Shirov [25] has shown that addition of strontium tungstate or phosphate, or of magnesium or beryllium tungstates, imparts a red or a violet tint, respectively, and K. Kamm [115] has prepared and described a range of zinc sulphide-cadmium sulphide phosphors by activation with copper and silver. This work has been influenced mainly by the requirements of the paint industry,[116] and is further discussed on page 330, and by Costeanu and St. Cocosinschi.[49] Zinc sulphide may be activated by an electric current (*cf.* G. Déchêne [111]) or by X-rays (*cf.* L. Levy and D. W. West [112]). The latter aspect of the subject has been studied extensively in connection with the production of screens suitable for counting α-particles emitted by radioactive preparations. Thus G. Destriau [113] has found that although a phosphorescent sulphide does not necessarily scintillate under the action of α-particles, and conversely the

fluorescence produced by the action of X-rays is very similar to the emission from the α-particle scintillations.

The use of 8-hydroxyquinoline as a test for zinc is referred to on page 298. M. Haitinger [59] mentions a microscopical adaptation of Danckwortt's macro-fluorescence method for the detection of zinc [60] (*e.g.*, in homeopathic triturates or in cinnabar), which is stated to be sensitive to 1 part in 10,000 ; reference should also be made to the section on activation and inhibition and to the work of A. A. Guntz [57] (see below).

Inhibition and Activation.[114]—These phenomena are discussed in connection with methods of technique on page 54, but as inorganic compounds provide many striking examples (see especially under zinc, uranium and the alkali and alkaline earth metals) they may be mentioned again here. It is unfortunate that so little is known concerning the mechanism of these effects, particularly as they are so intimately related with the presence of impurities in the substance concerned, and it must be admitted that the theories suggested (see Eisenbrand [50]) are, to say the least, inadequate.

So far as *inhibition* is concerned, uranium (p. 197) has proved a convenient subject for experiment. The work of Bouchard [45] has already been mentioned, and this worker has been able to derive a quantitative exponential expression for the intensity of fluorescence in terms of the concentration of the salt and a constant depending on its nature. Volmar and Mathis [53, 56] have even used the principle to detect ions which are powerful inhibitors (in the presence of less potent ions) from a knowledge of the limiting concentrations required to extinguish the fluorescence of a standard solution of 6 grms. of uranium acetate in 200 c.c. of 0·03 N sulphuric acid.[106] $Ag^{\cdot}$ was the only active cation, active anions (in order) being I' ($N/3000$), CNS', Br', $FeCy_6''''$, Cr_2O_7'', AsO_3''', S_2O_3'', CrO_4'', NO_2', $FeCy_6'''$ and Cl' ($N/75$) ; the activity of the halogen ions increases regularly with atomic weight. The concentration of the ion is plotted against the minimum number of drops required to suppress fluorescence, and a hyperbola is obtained. Addition of 0·5 to 2 parts per million of nickel to zinc sulphide to prevent the persistence of fluorescence after irradiation is the subject of a recent patent by the Siemens company.[55] H. Gotŏ [121] describes a similar method in which the change in fluorescence produced by the addition of each of

21 metal ions to a fluorescent substance is used as a means of identifying these ions.

Oxygen quenches the fluorescence of uranine on silica gel, and is thereby detectable [74, 75]; whilst Benoist [77] and Maché [78] have used successfully the variation in fluorescence of fluorescein under the action of *ozone* to determine the latter, quantitative applications of a similar reaction having been evolved by M. Schlesinger-Konstantinova,[94] and by W. Heller.[101] A test for oxygen is also provided by the fluorescence which results when a mixture of this gas and acetone vapour is exposed to filtered ultra-violet light; the test is best carried out by comparing the mixture side-by-side with the untreated sample under the lamp (W. F. Fulton [96] and G. H. Damon [109]). A solution of 2.7—diaminofluorene hydrochloride develops a blue fluorescence on exposure to oxygen in ultra-violet light (*cf.* p. 44).

With reference to *activation*, zinc may be cited as an example. In connection with the preparation of luminous paints it has been known for some time that certain salts (*e.g.*, of copper or manganese) act as " phosphorogens " or activators of fluorescence of *zinc sulphide*, but the investigations of A. Kutzelnigg [46] and R. Coustal [54] have shown that the conditions under which the phosphorogen is incorporated are also of importance; the latter fuses zinc sulphide in nitrogen under pressure at 2000° C. in the presence of 1 part in 10^5 of copper, and N. F. Shirov [64] includes boric acid and a silicate.

J. Ewles [16] has made the interesting observation that many white salts fluoresce with a bright blue colour when damp, but that this fluorescence disappears on drying. In most cases, there is also a phosphorescence which lasts for several seconds after the exciting light is withdrawn. The disappearance of the blue fluorescence on complete drying may indicate that water is an activator of luminescence, and may explain some of the deviations between results of different workers (*cf.* Table 12). Tiede [19] found that all pure specimens of magnesium sulphide fluoresce faintly on exposure to radiation from the sun or from an arc lamp, although they do not appear to respond to ultra-violet or X-rays alone.

Danckwortt [17] has applied the capillary-strip method (see p. 59) to the examination of *colloidal sols*, and K. Borgmann [61] has also studied the fluorescence of colloids. A strip obtained

from a red-violet gold sol gave an upper light blue zone and a lower reddish-purple zone. With a silver sol, the upper zone appears yellowish-brown and the lower reddish-purple in colour. A colloidal arsenic sulphide sol prepared according to de Haen's method, gave a faint yellow zone, and below this, a broad light blue zone merging into a dirty yellowish-brown band.

Rare earths (see also Minerals, p. 266). Tomaschek and Deutschbein [31] discuss their fluorescence spectra, and M. Haitinger [32] examined the marked fluorescence obtained when they were dissolved in borax or phosphate beads ; he also used a spectroscope and was able to obtain a sensitiveness exceeding that of absorption spectroscopy. The results are summarised below :—

Rare Earth.	Fluorescence of Bead.	Sensitiveness.	
		1 in	10^{-6} grm.
Cerium oxalate . .	Blue	10^4	0·5
Europium . . .	Red	500	25
Samarium . . .	Orange	1000	5
Gadolinium . .	Yellow	100	50
Terbium . . .	Yellow-green	5000	2·5
Dysprosium . .	Yellow	1000	5
Thulmium . .	Violet-blue	—	—
Holmium . . .	Pale yellow	—	—

The other cerite earths, lanthanum, praseodymium, neodymium, erbium, ytterbium and cassiopeium did not fluoresce. Europium develops a characteristic fluorescence spectrum if heated with an alkali halide.[72] The influence of rare earths on the fluorescence of glasses is discussed in Chapter IV, and in connection with mineralogy in Chapter XII ; see also Additional References.

Terbium.—A. Filippov, J. Larionov and A. Seidel [117] were able to detect traces of terbium in solution by means of the fluorescence of the $Te^{\cdot\cdot\cdot}$ ion ; the nature of the fluorescence depends on the duration of heating the solution before examination. Trivalent *europium* also fluoresces in solution, but when it occurs in minerals (*e.g.*, in fluorites) it may be reduced to the divalent state (*e.g.*, by the action of radioactive radiations), and it then fluoresces with a blue colour. *Gadolinium* produces similar effects, and is detectable in europium salts.

Samarium.—Similarly, K. Przibram [118] ascribed the yellow-green and red fluorescence of certain fluorites to divalent ytterbium and samarium or thulium, respectively, Certain of the rare earth elements (*e.g.*, samarium, europium and dysprosium) may be detected in concentrations of 1 p.p.m. from the spectrum of the luminescence excited by exposure to an electric discharge at a controlled temperature (*cf.* M. Servigne [119, 120]). With samarium in very dilute solid solution an accuracy of about 5 per cent. is obtainable for $1{\cdot}07 \times 10^{-4}$ to $0{\cdot}082 \times 10^{-4}$ grm. per gram of diluent (*e.g.*, calcium tungstate).

Rhenium.—B. Tougarinoff [42] found that 0·01 mgrm. (as perrhenate) could be detected from the yellow-red colour and, after heating, the green-yellow fluorescence, produced on adding 1 c.c. of sample to a mixture of 3 drops of a saturated solution of dimethyl glyoxime in alcohol, and 3 c.c. of a 25 per cent. solution of crystalline stannous chloride in concentrated hydrochloric acid.

Aluminium.—Goppelsroeder's test [41] has already been referred to in connection with foods, a yellow fluorescence being produced with tetrahydroxy flavonol (morin or fustic) even in the presence of 1 drop of a solution containing 0·3 γ of aluminium (see also beryllium). E. Tiede and H. Lüders [58] have studied the relation of the fluorescence and crystal structure of aluminium oxides. A. Okac [100] has attempted to make the morin reaction quantitative by titrating alkali fluorides with a solution of aluminium chloride containing morin, in the presence of sodium chloride; a fluorescence is produced when aluminium is no longer removed (as the AlF_6''' ion). If in this morin reaction sodium fluoride is first added, the green fluorescence of *gallium* is obtainable if this element is present, even if aluminium is present also (G. Beck [85, 99]). The thin layer formed on aluminium by electrolysis can be made to phosphoresce under suitable conditions (*cf.* H. Betz [87]), and since it is unaffected by the presence of most other metals (with the exception of zinc and manganese), there is the possibility that this might be adapted to analytical uses. Other tests for aluminium and notably that due to J. A. Radley [92] are referred to under Textiles (Chapter XVIII); another reaction (due to C. E. White and C. S. Lowe [88]), which may be used in the presence of beryllium, is the production of an orange-red fluorescence with 0·5 c.c. of a 0·1 per cent. solution of Pontachrome Blue Black R (the zinc salt of 4-sulpho-2,2′-dihydroxy

azonaphthalene) in the presence of acetic acid. Interfering elements should be removed by a preliminary precipitation with sodium hydroxide, and hexavalent chromium and fluorides should be absent. The sensitiveness is 0·2 p.p. million, but if less than 1 p.p. million of aluminium is present, the development of the fluorescence may take some time. Aluminium sulphate activated with eosin, erythrosin and similar dyes is phosphorescent[90] (*cf.* boric acid, p. 198).

Scandium and indium may also be detected by Beck's modification [99] of the morin reaction, because the former only fluoresces in the presence of an excess of ammonium carbonate. The reaction for indium is strongest in weakly acid solutions, but a negative result is obtained if the indium is removed by precipitation with hydrogen sulphide in the presence of sodium acetate as a buffer, the fluorescence of the other three elements being unaffected by this procedure. The sensitiveness is of the order of 0·02 to 1 γ per c.c., according to the conditions.

Molybdenum.—Tincture of cochineal produces a fiery-red fluorescence in solutions of molybdenum salts at *p*H 5·7 to 7·2 (L. Szebellédy and J. Jónás [95]). Lead, mercury, copper, bismuth, cobalt, nickel, iron, manganese and vanadates interfere; but silver, cadmium, arsenic, antimony, tin, zinc, ammonium tungstates and the other alkali and alkaline earth metals do not.

Summary.—At present it appears that the application of the lamp in technical inorganic work is very limited, but that after more work has been carried out on the influence of impurities on the fluorescence colour of various compounds, it may find some use in routine testing in commercial laboratories. Furthermore, its possibilities in the detection of small amounts of substances in biological, medical and forensic work have not yet been fully examined, and offer a promising field of investigation (see p. 223). One of us (J. Grant) has found, for example, that the sensitiveness of certain *colorimetric reactions* may be increased considerably (usually by about 10 times) if the matchings are carried out in ultra-violet light. This applies particularly to sulphide colours such as those produced from cadmium, bismuth, arsenic, lead and antimony. The method described by Grant [21] for antimony, for example, in which hydrogen sulphide water is added to an acid solution of the sample, and the yellow colour is matched against that of a standard, may be used to determine 0·0001 to 0·001 mgrm. of antimony in this way.

Similarly, L. T. Fairhall [43] and L. Prodan were able to determine 1 part of cadmium in 2,500,000 after wet oxidation, precipitation twice, and finally addition of hydrogen sulphide in the presence of potassium cyanide.

The most important results are summarised in the bibliography by M. Haitinger [63] and in Table 12.

TABLE 12.

FLUORESCENCE OF SOLID INORGANIC SUBSTANCES.

Substance.	*Fluorescence.*
Aluminium acetate.	Strong blue.
,, bromide.	Very bright red.
,, oxide.	Rose.
,, phosphate.	Blue-white.[30]
Ammonium salts.	Blue.
Arsenious tri-iodide.	Yellow-white.
,, trioxide.	Blue-white.
Barium acetate.	Bright green.
,, carbonate.	Red-brown.[26]
,, formate.	Bright violet shimmer.
,, pyrovanadate.	Light yellow.[11]
,, succinate.	Violet.
,, sulphide.	Intense red.[11]
,, sulphide (heated).	Orange to yellow.[11, 12]
Beryllium carbonate.	Violet.
,, oxide.	Violet.
Boric acid.	Blue-white.
Cadmium tungstate.	Strong yellow.
Cæsium sulphate.	Strong bright blue.
Calcium ammonium arsenate.	White shimmer.
,, phosphates.	Blue or violet.[30]
,, pyrovanadate.	Weak red-brown.[11]
,, salts (most).	Shades of blue.
Carbon dioxide (solid).	Yellow-blue.[46]
Cupric chloride.	Strong light green.[71]
,, iodide.	Violet.[11, 71]
Cuprous iodide (precipitated).	Violet.[46, 71]
,, ,, (washed).	Red.[46]
,, nitrate.	Violet.
Ferric phosphate.	Dark blue.[30]
Lead acetate.	Blue tinge.
bromide.	Sandy yellow.[11]
carbonate	Sandy yellow.
carbonate (basic).	Brighter yellow.
chloride.	Yellow-white shimmer.
sulphate.	Sandy yellow.
,, salts (most other).	None.[3]
Magnesium ammonium arsenate.	White shimmer.
,, oxide (in molten ammonium nitrate).	Scarlet.[46]
,, phosphate.	Strong blue.[30]
,, sulphate.	Violet-brown.[26]

TABLE 12.—*Continued.*

Substance.	*Fluorescence.*
Manganese acetate.	Red, white tinge.
bromide.	White.[3]
formate.	Strong light blue.
iodide.	Yellow.
oxalate.	Feeble white shimmer.
Mercurous chloride.	Red-pink.[33]
Potassium salts.	Usually violet.
Sodium salts.	Usually violet.
Sulphur.	Yellow-brown.[26]
Ultramarine.	Dark violet.[26]
Uranium salts.	Vivid yellow.[6, 7, 8, 49, 68]
Zinc carbonate (basic).	Weak blue-violet.[13, 49]
hydroxide.	Blue-violet.[13]
oxalate.	Strong green (colourless if pure).[14, 15]
oxide.	Yellow-green.[68]
potassium cyanide.	Blue-violet.
sulphide.	Yellow or orange to green according to purity. Inhibited by Fe or Co but not by Pb[20], and stimulated by Cu[22].
Zirconium oxide.	White phosphorescence.[27]

The following compounds have no appreciable fluorescence in the pure state :—

Antimony trichloride. Arsenic trisulphide. Barium bromide and thiosulphate. Bismuth iodide and oxide. Cadmium carbonate and sulphate. Cæsium nitrate. Calcium chloride, iodide and nitrate. Chromic acid, chromium trichloride and trifluoride. Cobalt nitrate and sulphate. Copper oxalate, sulphate and thiocyanate (cupric). Lead chromate and oxides (mono- and di-) and most other lead salts. Ferric chloride, oxide and thiocyanate. Ferrous sulphate. Magnesium sulphate. Manganous chloride. Mercuric iodide and sulphide. Mercurous chromate, iodide and oxide. Nickel chloride and oxide. Potassium carbonate. Sodium biborate, hypophosphite, nitrate, phosphate (di-acid), sulphide, thiosulphate. Sulphur (flowers). Vanadium trioxide. Zinc carbonate, chloride, chromate, nitrate, oxalate, phosphate, silicate, sulphate and zinc green.[14, 15]

REFERENCES.

1 H. Lehmann, *Phys. Zeit.*, 1912, **13**, 35.
2 O. Wolf, *Chem. Ztg.*, 1912, **36**, 197, 1039.
3 W. Lenz, *Zeit. anal. Chem.*, 1915, **54**, 27.
4 A. A. King, *Chem. and Ind.*, 1928, **4**, 301.
5 J. Papish and L. E. Hoag, *Proc. Nat. Acad. Sciences*, 1927, **13**, 726.
6 E. Nichols and M. K. Slatterly, *Jour. Opt. Soc. Amer.*, 1926, **12**, 449.
7 H. Eitel, *Arch. Exp. Path. u. Pharmacol.*, 1928, **135**, 188.

[8] F. Perrin, *Compt. rend.*, 1928, **186**, 428.

[9] N. F. Shirov, *Zhur. Prikladnoi Khim.*, 1930, **3**, 675.

[10] J. Donau, *Monatsh. f. Chem.*, 1913, **34**, 949.

[11] R. Robl, *Zeit. angew. Chem.*, 1926, **39**, 608.

[12] W. S. Andrews, *General Elec. Rev.*, 1925, **28**, 659.

[13] E. Tiede and F. Goldschmidt, *Ber.*, 1929, **62**, 758 ; *Mikrochem.*, 1932, **12**, 269.

[14] E. Beutel and A. Kutzelnigg, *Monatsh. f. Chem.*, 1931, **57**, 9, 15.

[15] E. Beutel and A. Kutzelnigg, *ibid.*, 1931, **55**, 158.

[16] J. Ewles, *Nature*, 1930, **125**, 706.

[17] P. W. Danckwortt and E. Pfau, *Analyst*, 1927, **52**, 707.

[18] H. Demassieux and V. Henri, *Compt. rend.*, 1931, **193**, 591.

[19] E. Tiede, *Ber.*, 1916, **49**, 1745.

[20] M. Curie and J. Saddy, *Compt. rend.*, 1932, **194**, June 6.

[21] J. Grant, *Analyst*, 1928, **53**, 626.

[22] J. Einig, *Chem. Ztg.*, 1932, **56**, 185.

[23] A. Wakenhut, *Farben. Chem.*, 1931, **2**, 454.

[24] L. Vanino and S. Rothschild, *Chem. Ztg.*, 1932, **56**, 642.

[25] N. F. Shirov, *Jour. Appl. Chem., Russia*, 1932, **5**, 177.

[26] J. Muir, *Ind. Chem.*, 1933, **9**, 51.

[27] E. Holzinger, *Chem. Ztg.*, 1932, **56**, 1022.

[28] R. Thomaschek and O. Deutschbein, *Nature*, 1933, **131**, 472.

[29] M. Haitinger, F. Feigl and A. Simon, *Mikrochemie*, 1931, **10**, 117.

[30] F. Mach and P. Lederle, *Phosphorsäur.*, 1932, **2**, 623.

[31] R. Thomaschek and O. Deutschbein, *Nature*, 1933, **131**, 473 ; *Physikal. Zeit.*, 1933, **34**, 374.

[32] M. Haitinger, *Sitzungsber. Akad. Wiss., Vienna*, 1933, **142**, 339.

[33] F. W. Martin, *Analyst*, 1934, **59**, 531.

[34] I. Plotnikov, *Phot. Korr.*, 1932, **68**, 211.

[35] E. Becquerel, " La Lumière " (Paris, 1867).

[36] A. Grumbach and M. Ribaillier, *Compt. rend.*, 1934, **198**, 70.

[37] S. Oxa and S. Yagi, *Jour. Soc. Chem. Ind., Japan*, 1933, **36**, 143B.

[38] H. L. J. Zermatten, *Proc. Kon. Akad. Wetenschr., Amsterdam*, 1933, **36**, 899 ; through *Brit. Chem. Abst. A.*, 1934, 269.

[39] S. Satoh, *Nature*, 1934, **133**, 837.

[40] F. Hahn, *Compt. rend.*, 1933, **197**, 245.

[41] F. Goppelsroeder, *Zeit. anal. Chem.*, 1868, **7**, 195 ; F. Feigl, " Qualitative Analyse mit Hilfe von Tüpfel-reaktionen " (Leipzig : Akad. Verlagsgesell., 1938).

[42] B. Tougarinoff, *Bull. Soc. Chim. Belg.*, 1934, **43**, 111.

[43] L. T. Fairhall and L. Prodan, *Jour. Amer. Chem. Soc.*, 1931, **53**, 1321.

[44] F. Swindells, *Jour. Opt. Soc. Amer.*, 1933, **23**, 129.

[45] C. Achard, A. Boutaric and J. Bouchard, *Compt. rend.*, 1933, **196**, 485, 1317, 1757.

[46] A. Kutzelnigg, *Zeit. anorg. Chem.*, 1932, **208**, 29 ; *Monatsh.*, 1934, **64**, 74.

[47] R. E. Lutz, *Jour. Ind. Hyg.*, 1925, **7**, 293.

[48] E. Beutel and A. Kutzelnigg, *Monatsh. Chem.*, 1932, **61**, 69 ; *Sitzungsber. Akad. Wiss., Vienna*, 1932, **141**, 437 ; *Chem. Ind.*, 1933, **52**, 404 ; A. Kutzelnigg, *Zeit. anorg. Chem.*, 1932, **208**, 29 ; 1935, **223**, 251 ; *Monatsh.*, 1937, **70**, 297.

[49] N. D. Costeanu and A. St. Cocosinschi, *Bull. Fac. Stünte Cernauti*, 1931, **5**, 169 ; through *Brit. Chem. Abst. A.*, 1934, 346.

[50] J. Eisenbrand, *Zeit. physikal. Chem.*, 1933, **22B**, 145.

[51] D. Ganassini, *Chem. Zentr.*, 1904, (i), 1172.
[52] L. Szebellédy and J. Gaál, *Magyar Gyógyszerésztud Tarsasag Ert.*, 1934, 10, 309 ; *Zeit. Anal. Chem.*, 1934, 98, 255.
[53] Y. Volmar and V. Mathis, *Bull. Soc. Chim.*, 1933, **53**, 385.
[54] R. Coustal, *Compt. rend.*, 1933, **196**, 1306 ; 1934, **198**, 1403, 1596.
[55] Siemens-Reinger-Werke, A.G., B.P. 406,957, 8/11/33.
[56] Y. Volmar and V. Mathis, *Bull. Soc. Chim.*, 1933, **54**, 1266.
[57] A. A. Guntz, *Compt. rend.*, 1933, **197**, 1030.
[58] E. Tiede and H. Lüders, *Ber.*, 1933, 66B, 1681.
[59] M. Haitinger, *Mikrochemie*, 1932, **11**, 429.
[60] P. W. Danckwortt, *Apothekerztg.*, 1929, No. 81.
[61] K. Borgmann, *Sudd. Apoth. Ztg.*, 1931, **71**, 70.
[62] L. Szebellédy and H. Gaál, *Zeit. Anal. Chem.*, 1934, **98**, 255.
[63] V. V. Romanov, *Comp. rend. Acad. Sci., U.S.S.R.*, 1934, **3**, 432.
[64] N. F. Shirov, *Jour. Appl. Chem. Russ.*, 1934, **7**, 343.
[65] H. R. Fleck, R. F. G. Holness and A. M. Ward, *Analyst*, 1935, **60**, 32.
[66] H. Eichler, *Zeit. Anal. Chem.*, 1934, **99**, 272.
[67] H. Eichler, *ibid.*, 1934, **99**, 270.
[68] M. Haitinger, *Mikrochemie*, 1935, **16**, 321.
[69] N. F. Shirov, *Jour. Appl. Chem., Russia.* 1934, **7**, 921.
[70] H. Eichler, *Zeit. Anal. Chem.*, 1935, **100**, 183.
[71] J. Terrien, *Compt. rend.*, 1935, **200**, 1096.
[72] K. Przibram, *Acad. Sci. Vienna*, 7/3/35.
[73] N. F. Shirov, T. E. Oetman and E. J. Matenko, *Jour. Appl. Chem., Russia*, 1934, **7**, 1382.
[74] H. Kautsky and A. Hirsch, *Zeit. Anal. Chem.*, 1935, **222**, 126 ; *Trans. Faraday Soc.*, 1939, **35**, 216.
[75] G. H. Damon, *Jour. Ind. Eng. Chem.*, 1935, **7**, 133.
[76] E. Schneider, *Zeit. physikal. Chem.*, 1935, B, **28**, 311.
[77] L. Benoist. through *Analyst*, 1919, **44**, 183.
[78] A. Maché, *Compt. rend.*, 1935, **200**, 1760.
[79] W. M. Seaber, *Analyst*, 1936, **61**, 14.
[80] J. Grant, *ibid.*, p. 400.
[81] F. L. Hahn, *Mikrochem.*, 1935, **17**, 222 ; *Compt. rend.*, 1935, **201**, 296.
[82] J. Grant, *Analyst*, 1937, **62**, 285.
[83] R. Gros, *J. Pharm. Chim.*, 1935, **22**, 211.
[84] H. L. Hahn, *Mikrochem.*, 1936, **20**, 236.
[85] G. Beck, *ibid.*, p. 194.
[86] G. Bertrand and L. de Saint-Rat, *Compt. rend.*, 1936, **203**, 140.
[87] H. Betz, *Z. Physik*, 1935, **95**, 189.
[88] C. E. White and C. S. Lowe, *Ind. Eng. Chem. Anal.*, 1937, **9**, 430.
[89] L. Szebellédy and S. Tanay, *Z. Anal. Chem.*, 1936, **107**, 26.
[90] V. L. Levschin and L. A. Vinokurov, *Physik. Z. U.S.S.R.*, 1936, **10**, 10.
[91] W. Langenbeck and U. Ruge, *Ber.*, 1937, **70B**, 367 ; O. Schales, *ibid.*, 1938, **71B**, 447.
[92] J. A. Radley, *Analyst*, 1938, **63**, 266.
[93] G. Bertrand and L. de Saint-Rat., *Mikrochim. Acta*, 1937, **1**, 4.
[94] M. Schlesinger-Konstantinova, *Compt. rend. Acad. Sci. U.S.S.R.*, 1937, **14**, 187.
[95] L. Szebellédy and J. Jónás, *Mikrochim. Acta*, 1937, **1**, 46.
[96] W. F. Fulton, *Oil and Gas J.*, 1937, **36**, 60, 62.
[97] L. Rosenthaler, *Mikrochem.*, 1937, **23**, 194.
[98] L. A. Biggs, *J. Ind. Hyg.*, 1938, **20**, 161.

[99] G. Beck, *Mikrochim. Acta*, 1937, **2**, 287.

[100] A. Okac, *Coll. Czech. Chem. Comm.*, 1938, **10**, 177.

[101] W. Heller, *Compt. rend.*, 1935, **201**, 139.

[102] S. N. Roy, *J. Ind. Chem. Soc.*, 1936, **13**, 40.

[103] S. Satoh, *Sci. Paper Inst. Phys. Chem. Res. Tokyo*, 1936, **29**, 41.

[104] F. Hernegger and B. Karlik, *Sitzungsber. Akad. Wiss. Wien*, 1935, **144**, 217.

[105] E. H. Nichols and H. L. Howes, " Fluorescence of the Uranyl Salts," Washington, 1905.

[106] H. Gotō, *J. Chem. Soc. Japan*, 1938, **59**, 547.

[107] S. Perrin and R. Delorme, *Compt. rend.*, 1928, **186**, 428.

[108] A. Schleede, *Chem. Ztg.*, 1935, **59**, 645.

[109] G. H. Damon, *Ind. Eng. Chem. Anal.*, 1935, **7**, 133.

[110] N. Z. Andreev, *J. Appl. Chem. Russ.*, 1935, **8**, 49.

[111] G. Déchène, *Compt. rend.*, 1935, **201**, 139.

[112] L. Levy and D. W. West, *Brit. J. Radiology*, 1935, **8**, 184.

[113] G. Destriau, *J. Chim. Phys.*, 1936, **33**, 587.

[114] Faraday Soc. (Discussion on Luminescence), *Nature*, 1938, **142**, 779.

[115] K. Kamm, *Ann. Physik*, 1937, **30**, 333.

[116] L. Vanino, *Chem. Ztg.*, 1935, **59**, 656.

[117] A. Filippov, J. Larionov and A. Seidel, *Compt. rend. Acad. Sci. U.S.S.R.*, 1936, **1**, 253 ; 1936, **3**, No. 3 ; 1937, **16**, 443.

[118] K. Przibram, *Nature*, 1937, **139**, 329 ; *Mikrochim. Acta*, 1938, **3**, 68.

[119] M. Servigne, *Compt. rend.*, 1936, **203**, 1247 ; 1937, **204**, 863.

[120] M. Servigne and E. Vassy, *ibid.*, 1937, **204**, 1566.

[121] H. Gotō, *J. Chem. Soc., Japan*, 1938, **59**, 547.

[122] E. Föyn, B. Karlik, H. Pettersson and E. Rona, *Nature*, 1939, **143**, 275.

[123] H. Herrmann, *Mikrochim. Acta*, 1938, **3**, 193.

ADDITIONAL REFERENCES.

O. Baudisch and F. Benford, *Naturwiss.*, 1936, **24**, 316.

A. Terenin and F. Clement, *Acta Physicochim. U.S.S.R.*, 1934, **1**, 941 (Fluorescence of salts which have been surface-activated by condensed metals).

H. Döring, *Naturwiss.*, 1935, **23**, 19 (Fluorescence which occurs on crystallisation).

F. G. Wick and C. G. Throop, *Jour. Opt. Soc. Amer.*, 1935, **25**, 57 (Activation of thoria by rare earths).

L. Levy, *Chem. Trade J.*, 1936, **98**, 191 (Fluorescent compounds for cathode tubes).

J. Weiss, *Nature*, 1936, **138**, 80 (Photo-reduction of fluorescent substances by ferrous ions).

J. A. Chvostikov, *Physik. Z. U.S.S.R.*, 1936, **9**, 210 ; *Trans. Opt. Inst Leningrad*, 1937, **12**, 3 (Fluorescence of platinocyanides).

J. V. Golbreich and I. V. Egorova, *J. App. Chem. Russ.*, 1936, **9**, 446 (Activation of zinc salts with manganese).

R. Servigne, B.P. 485,373, 13/11/36 (Activation of the alkali tungstates).

J. H. Hibben, *Phys. Rev.*, 1937, **51**, 530 (Coloration of crystals of magnesium oxide in ultra-violet light).

Degea, A-G., B.P. 485,232, 22/3/37 (Activation of zinc silicate by manganese).

M. Travničkek, *Ann. Physik*, 1937, **30**, 224 (Activation of magnesium cements by fluorescein, etc.).

R. Thomaschek and E. Mehnert, *Ann. Physik*, 1937, **29**, 306 (Fluorescence of solid gadolinium salts).

O. Deutschbein and R. Thomaschek, *ibid.*, p. 311 (Fluorescence of solutions of rare earths).

H. Gobrecht and R. Thomaschek, *ibid.*, p. 324 (Detection of rare earths from their fluorescence spectra).

H. Gobrecht, *ibid.*, 1938, **31**, 181 (Fluorescence of cerium, and of thulmium in the borax bead test).

J. T. Randall, *Nature*, 1938, **142**, 113 (Fluorescence of inorganic compounds at low temperatures).

W. H. Byler, *J. Amer. Chem. Soc.*, 1938, **60**, 632, 1247 ; N. Riehe, *Ann. Physik*, 1937, **29**, 636 ; *J. Phys. Radium*, 1938, **9**, 57D. (Effect of heat and physical characteristics on the fluorescence of zinc and other compounds.)

S. Rothschild, *Z. Physik*, 1938, **108**, 24 (Effect of cadmium and other metal sulphides on the fluorescence of zinc sulphide).

C. E. White, *Ind. Eng. Chem., Anal.*, 1939, **11**, 63 (General).

CHAPTER IX.

LEATHER AND TANNING.

VERY little work has been done on the actual examination of leather itself, although recently E. R. Theis and E. J. Serfass[45] have demonstrated the advantages of using light of wave-length 3650 Å. for studying sections under the microscope; they conclude that this leads to improved resolving power, permitting a better study of the fine structures present, and that the primary fluorescence allows the distribution of the various constituents, notably the oils and fats, to be more closely followed. By means of secondary fluorescence effects they found that the changes taking place during liming, soaking and bating could be followed.

By staining with Phosphine G (1 : 5000), Geranine GL (1 : 10,000) or Thioflavine S (1 : 100,000), and using dark-field ultra-violet illumination, they were able to show that liming effects the destruction of the stratum germinatium, that the hyaline layer is present after liming but is destroyed during bating, and that bating results in a partial destruction of the elastin fibres. To show the same changes (although much less clearly) with visible light, requires the application of several different stains. The use of the fluorescence microscope is also dealt with by A. Salmony-Karsten.[41,42]

Quite recently a number of *artificial tanning agents* (see p. 217) have been introduced into the leather industry with the result that the chemist is now called upon to distinguish these from natural tanning agents, and from mixtures of the two. Almost simultaneously several workers applied the use of the ultra-violet lamp to this problem, the work of L. Meunier[4] and O. Gerngross[7-15] being particularly noteworthy (see also Deforge [38]). L. Meunier and A. Bonnet [1] found that most natural tanning agents, such as oak, mimosa and chestnut barks, wort tree, mangrove, sumach and gall apples, etc., give practically no fluorescence under the

lamp, although freshly-cut surfaces of quebracho from South America, and tisera from Morocco, gave a brilliant yellow fluorescence. These workers identified the fluorescing substance in catechin tans with fisetin, thus confirming the work of Gunnell and Perkin,[2] who identified the colouring matter of quebracho colorado with the fisetin in fustic. Extracts of plants containing berbèrine (*e.g.*, calumba root) give a yellow fluorescence which is removed by addition of mercury potassium iodide ; this, of course, precipitates the berberine. The leaves, bark, wood, roots, twigs and cortex of the roots of Sicilian tisera have been examined by G. A. Bravo,[44] who found in this way that the tans present are exclusively of the catechol type.

Aqueous extracts of *quebracho* and *tisera* show only a slight fluorescence, but if various fibrous materials such as cotton-wool or filter-paper are dipped in the solutions and then placed under the lamp, a brilliant yellow-coloured fluorescence is observed, the maximum sensitiveness of the test being in the neighbourhood of 1 in 10,000. In this manner quebracho, both sulphited and unsulphited, and tisera, can be detected in mixed tan liquors and also in leather itself (*vide infra*). Mimosa, gambier and catechu treated in this manner show at first a violet fluorescence which fades, and finally changes to a yellow colour paler than that given by quebracho. Orchil has a weak, white fluorescence.

Violet fluorescence is characteristic of acetone extracts of the *pyrogallol tans*, *e.g.*, chestnut bark, oak bark, myrobalan, valonia, divi-divi, urunday [3] and the various galls. A blue-violet or blue-white fluorescence is given by oak bark and gonakie, which contain both pyrogallol and catechin tans, whilst leaf-tanning agents such as sumach, *Pistacia lentiscus* and *arbutus* show the dark red fluorescence of chlorophyll, which passes into the acetone. Dried extracts of the leaves do not show this effect.

Technique.—L. Meunier and A. Jamet [4] recommend the following procedure for the examination of tanning materials. The tanning agent, the dried powdered residue from the evaporation of an aqueous extract, or if a tanned leather is under examination the freshly-cut shavings, is shaken several times with 5 c.c. of acetone. The original extract should be as concentrated as possible, and then diluted by addition drop by drop to pure acetone. A method for differentiating between catechu, gambier and kino is due to H. Freytag,[31, 36] who adds potassium ferro-

cyanide and sodium nitrate to the extracts and examines the products under the lamp.

W. Appelius and L. Keigueloukis [5] have devised an alternative method for the identification of tanning agents in which the material (2·5 grms.) is heated under a reflux condenser with 20 c.c. of a 1 per cent. solution of antipyrine and 30 c.c. of 10 per cent. hydrochloric acid. Cotton-wool is then dipped into the solution, washed and examined under the lamp, when the typical fluorescent colours are visible. The method of fixing the tannins on cotton-wool and other fibres has been applied by G. Desmurs [6] who uses calico or Mulhouse strips. On the Mulhouse strips a yellow fluorescence is obtained with quebracho, tisera, algaroba wood, berberry, Japanese coptis or Colombe root. A fainter yellow fluorescence is obtained with hemlock, mimosa or true cutch, and violet with quassia wood, guaiacum and quillaia bark. L. Pollak [35] has examined vegetable and synthetic tanning agents by fixing them simultaneously on various textile fibres woven into the same piece of cloth and examining the fluorescence under the lamp. The most suitable fabric consists of wool with natural silk and mercerised cotton threads in the warp, and rayon and cotton threads in the weft. Pieces of this cloth 5 × 5 cm. are dyed on the water bath for 30 minutes in 50 c.c. of a 2 to 4 per cent. solution of the sample, in the presence of 2 c.c. of 2 per cent. acetic acid. The squares are then washed, dried and examined under the lamp when the effect seen is that of a shining lattice on the woollen background, the colours of the lattice varying with the agent examined.

O. Gerngross and his fellow-workers [7-15] found that the majority of tanning agents show only very little fluorescence in neutral solution, exceptions being tisera and quebracho, the fluorescence of which is repressed in both cases by addition of acid or alkali. The fluorescence of pine and malet is materially increased by treatment with alkaline salts, the blue changing to a characteristic grass-green colour, whilst donga extract changes from a red to a striking orange-red.

The violet fluorescence of *pine bark* extracts is due to a compound having the empirical formula $C_{33}H_{33}O_{13}$, which loses its fluorescence on acetylation. Pine or hemlock in acetone solution shows a fluorescence similar to that of mimosa or of gambier which, although it is weaker, is nevertheless distinctly visible.

Cotton-wool immersed in pine or malet extract gives a striking violet colour, which is also shown, though to a lesser extent, by oak bark extract. As a result of mixing materials which fluoresce yellow (*e.g.*, quebracho) and violet (*e.g.*, pine), a snow-white fluorescence is obtained. Mangrove bark extract also shows a weak white fluorescence on cotton-wool, and although it is classed as a catechol tan the yellow tints are only very faintly apparent; it therefore appears to be an exception to the general rule.

Fisetin.—The yellow fluorescence in ultra-violet light shown by quebracho, tisera and urunday is known to be due to the fisetin present, and F. V. Lutati [32, 37] has examined a number of woods and noted a fluorescence from several not previously recorded, *e.g.*, *Leguminosæ*, *Gleditschia triacanthos*, *Acacia farnesiana*, *Cercis siliquastrum*, *Ceratonia siligua*, among *Anacardiaceæ*, *Pistacia vera*, among *Rhamnaceæ*, and *Zisyphus vulgaris*. This worker found that there was no connection between the tannin content and the fluorescence, *e.g.*, *Gleditschia* is free from tannin. He observes that aqueous solutions of fisetin are practically non-fluorescent, but when fixed on an adsorbent it becomes highly fluorescent; this, he considers, is due to the degree of dispersion. Solutions of this compound in ethyl or amyl alcohol, ether, acetone, chloroform, benzene or ethyl acetate are fluorescent.

The difficulty in dealing with mixtures of substances which together show a white fluorescence but separately appear yellow and violet, has been overcome by O. Gerngross [15] who found that nitrocellulose which has not been too highly nitrated (11 per cent. of N) absorbs selectively the yellow-fluorescing fisetin but not the substance responsible for the violet colour. Further, on the assumption that it is only the fisetin in the fibre which produces a yellow fluorescence, O. Gerngross and H. Hübner [11] have been able to estimate the fisetin content of solid quebracho extract as 15 to 17 per cent., and on this basis they have evolved a method in which the extracts are diluted until no further weakening of the fluorescence occurs, the tan being fixed on cotton-wool. The fluorescence of a quebracho extract fixed on cotton-wool is stated to be still clearly visible in dilutions of 1 in 1,000,000, and the percentage of quebracho present in an extract may therefore be obtained approximately from the maximum dilution at which the fluorescence is just visible.

V. Kubelka and V. Němec [16] examined the solvent layer

obtained on shaking 20 c.c. of extracts of various tanning materials in water with 10 c.c. of ether or ethyl acetate for 5 minutes. They also examined the fluorescence from acid, neutral and alkaline extracts of the tannins fixed on cotton-wool or on filter-paper. As a test for small amounts of gambier in admixture with other tannins, they shake with 5 c.c. of alcohol, and examine the liquid under the lamp. It shows a grey-violet fluorescence, which develops a faint green tinge on addition of 1 c.c. of sodium hydroxide solution. The alcoholic mixture is then extracted with 10 c.c. of petroleum spirit which, on subsequent separation, shows under the lamp a brilliant pale green fluorescence in the presence of small quantities of gambier.

Synthetic Tanning Agents.—Many artificial tanning agents show an extremely strong and, in general, a characteristic fluorescence. In admixture with the natural tanning agents their fluorescence is, however, considerably diminished, but in most cases 10 to 20 per cent. can be detected with certainty. O. Gerngross, G. Sándor, and K. Tsou [13] have examined the absorption of various fluorescent substances contained in quebracho and pine bark extracts, and furthermore, have clarified the relation between chemical combination and adsorption.

O. Gerngross,[17, 18] in his studies on the fluorescence of artificial tanning agents, showed that sulphite cellulose and its lyes have a characteristic bright violet fluorescence which changes to green in alkaline solution. "Tannesco," "Ordoval," and "Carpatan" -*A* or -*R*, which give no positive reaction with the aniline-hydrochloric acid reagent, appear strongly blue or bluish-violet even in high dilutions. G. Desmurs [6] has shown that the synthetic tannins derived from naphthalene or anthracene have a deep violet fluorescence ; quinol appears pale violet and phloroglucinol sky-blue. He also mentions the white fluorescence shown by orchil, alkanet, and mixtures of mimosa with pyrogallol tan or urunday. According to L. Meunier and A. Jamet [19] the synthetic tans cannot be distinguished in this way from sulphite cellulose extract, but synthetic tannins are too expensive to be used as adulterants of the latter. Some synthetic tannins (*e.g.*, "maxyntan") have no fluorescence. H. Kimreuther, E. Schlumberger and W. Nippe [20] showed that unbleached sulphite cellulose substances had similar violet fluorescences, and also that these changed in colour to green on addition of an alkali.

The observation of fluorescence in ultra-violet light provides an easy method to follow the decomposition of technical *sulphite cellulose* (*cf.* p. 340). The last-mentioned workers ascribe the fluorescence to lignosulphonic acid, and since W. Leupold [21] attributes it to colloidal sulphur, it is probable that the fluorescence is due to sulphur compounds. In support of this view Leupold has suggested that iodine and chlorine, which destroy the fluorescence, would also probably destroy any thio- or polythionic acids. Later E. Hägglund and T. Johnson,[22, 23] and also O. Gerngross and his fellow-workers (*loc. cit.*), studied the lignosulphonic acid theory exhaustively, and confirmed it in its essential details, and in this they are in agreement with the conclusions of B. Rassow and G. Brandau.[24] Sulphite cellulose in commercial tan extracts may be detected by the characteristic fluorescence obtained when three drops are added to 10 c.c. of N potassium hydroxide solution (Meunier and Le Viet,[28] Cuccodoro [29] and Auerbach [30]). According to A. Noll,[48] the minimum quantity of ligno-sulphonic acid detectable by the fluorescence method is 63 γ in a dilution of 1 : 48,000 (see pp. 280 and 340).

A. Karsten [42] considers that 10 to 20 per cent. of syntans are detectable in natural tans. Sulphite cellulose shows a characteristic purple fluorescence which shades into green in alcoholic solution, but if it is suspected in natural tans, these must first be precipitated by means of sodium potassium tartrate and lead acetate. Spruce and mallee bark have a strong purple fluorescence which is not removed by this treatment, so that the test is invalid in their presence. The determination of the pH value of synthetic tanning agents has been carried out by A. Borgialli [43] who used Thioflavine, Patent Phosphine and Acid Phosphine R as indicators for titrating solutions of syntans, and he found that even weak acid gave a very sharp end-point, no interference by the fluorescence of the syntans themselves being experienced. Some of the various synthetic tanning agents on the market have been examined by Grassmann and Lang by the chromatographic method (see below), and they find that each has its characteristic number and disposition of coloured zones which allow them to be differentiated.

Table 13 is compiled from the results of several workers on the subject. The first three columns show the fluorescence

of the tan extract originally and after addition of acid and alkali, respectively, and are due mainly to L. Pollak and W. Springer.[26] The results shown in the fourth column were obtained by C. van der Hoeven [25] after the extraction of 1 grm. of tan at 60° C.

TABLE 13.

Extract.	Direct.	Acid.	Alkaline.	Ethyl Acetate.
Acacia	Brown	Brown	Brown	—
Algaroba	Purple	Brighter	Duller	—
Catechu	Purple	Lilac	Brown	—
S. African Catechu	Yellow	Brighter	Dirty violet	—
Chestnut	Very weak violet	Brighter	Dull brown	Nil
Divi-divi	Brown	Brighter	Duller	—
Elm bark	Intense purple	Yellow-brown	Bright yellow-brown	—
Gallic acid	Violet	Violet	Yellow	—
Gambier	Violet	Brighter	Yellow-green	Rose
Hemlock	Bright violet	Bright violet	White or dirty green	Nil
Mangrove	Intense purple	Bright Violet	Orange	Rose
Mimosa	Violet	Brighter violet	Violet to brown	Rose
Myrobalan	Purple	Bright violet	Dark purple	Yellow
Oak (wood)	Weak brown-violet	Violet	Violet	Nil
Pine (bark)	Lilac	Lilac	Dirty green	Yellow to rose
Pistacia	Yellow	Yellow	Dirty violet	—
Pyrogallol	Violet	Violet	Purple	—
Quebracho	Green-yellow	Bright yellow	Violet	Rose
Sulphite Cellulose	Violet	Violet	Intense yellow	Red
Sumach	Violet	Bright purple	Dark purple	Yellow
Urunday	Violet	Bright violet	Dirty yellow	Red
Valonia	Green-violet	Bright violet	Dark purple	Yellow
Willow (bark)	Violet-blue	Yellow-green	Violet	—

with 10 grms. of sodium hydroxide solution, the extract being then neutralised with 80 per cent. sulphuric acid and diluted to 500 c.c. After filtration 100 c.c. of the mixture were shaken with 50 c.c. of ethyl acetate and the filtered extract examined.

G. Desmurs [33] has used the lamp to differentiate between leather that has been tanned exclusively with bark and by the more rapid method of tannage with quebracho. The fluorescence is not very strong with pure vegetable-tanned leather, but is accentuated considerably when the leather has been mordanted with chrome. Like Grasser [27] he uses the lamp for the detection of oil, and further, claims to be able to identify the oil. Grassmann and Lang [40] have applied the methods of capillary analysis and of adsorption on silica gel.

Their method, which appears capable of considerable extension, has been described more fully on page 60. The chemical tanning agents such as phenol, resorcinal, tannic acid, etc., show only one zone in the adsorption tube, whereas the vegetable tanning agents show a variety of coloured zones. The following are the results obtained by these workers when using solutions in aqueous methyl alcohol with aluminium oxide as the adsorbing agent, the figures giving the depth of the appropriate zone in mm. :—

Oak wood.	Dark, 30 ; Bright greenish-blue, 70.
Chestnut wood.	Dark brown green, 40 ; light blue, 11.
Sumac.	Dark greenish-brown, 80 ; yellow-green, 3 ; light blue, 7.
Myrobalan.	Dark olive green, 60 ; dark, 20 ; bluish-white, 2 ; weak yellow, 14 ; weak blue-grey, 10.
Valona.	Dark greenish-brown, 50 ; sulphur yellow, 30 ; bright grey, 20.
Algarobilla.	Dark greenish-brown, 50 ; bright yellow, 5 ; dark steel-grey, 40.
Gambier.	Brown, 25 ; yellow, 40 ; bright greenish-blue, 24 ; grey brown, 7.
Quebracho.	Ochre, 50 ; brown, 8 ; whitish-yellow, 2 weak bluish ring.
Mimosa.	Whitish red-brown, 55 ; sky blue, 2 ; bright ochre, 13 ; bright blue, 40.
Willow bark.	Greenish-brown, 17 ; blue-violet, 30 ; bright greenish-blue, 65.
Tisera.	Greenish-brown, 50 ; dirty pale bluish-green.

These workers have also examined a number of synthetic tanning agents by this method, and also the resins extracted from

pine bark by means of benzol; the chromatogram of the latter shows no less than 11 different coloured zones of varying depths. An improvement on Grassmann's method is due to L. Pollak and A. Patzenhauer [46] who obtained similar results more conveniently by examining the tanned hide-powder residue and the cotton-wool pad obtained in the filter-bell method of tannin analysis. The division of the colours is not so well defined, but the necessity for an adsorbent such as alumina is elimated.

Jovanovits and Sampson [39] used the lamp, in conjunction with the microscope, to determine the degree of penetration of tanning agents into the leather from the typical changes in the fluorescence of the original skin due to tanning. E. R. Theis and E. J. Serfass [47] have developed this method by applying the principles of fluorescence microscopy. The fluorescence of oils may be used to study penetration phenomena associated with fat liquoring and oiling-off; and foreign substances in leather, which may be missed by the usual microscopical methods, are also often detectable. Dyes such as Geranine-GL, Phosphine-G or Thioflavine-G (1 : 5000 to 1 : 100,000) enable constituents such as elastin, collagen, sebaceous glands, hairs, epidermis, etc., to be seen, and the presence of the hyaline layer and its subsequent decomposition during bating is apparent. Other changes in structure which occur during the tanning operations may be studied in this way (*cf.* p. 213).

Although not strictly under the heading of leather or tanning, mention may here be made of the fluorescence of the various Ursol colours present in furs. A. Goldberger [34] extracts the dye by means of various solvents and examines the fluorescence of the solutions under the lamp (*cf.* Chapter XX).

REFERENCES.

1 L. Meunier and A. Bonnet, *Compt. rend.*, 1925, **180**, 2038; **181**, 465.

2 A. G. Perkin and O. Gunnell, *Jour. Chem. Soc.*, 1896, **69**, 1303.

3 W. Vogel, *Collegium*, 1926, 535.

4 L. Meunier and A. Jamet, *Jour. Int. Soc. Leather Trades Chem.*, 1926, **10**, 166; *Cuir Tech.*, 1926, **19**, 165.

5 W. Appelius and L. Keigueloukis, *Collegium*, 1930, 330.

6 G. Desmurs, *Jour. Int. Soc. Leather Trades Chem.*, 1929, **13**, 616.

7 O. Gerngross, N. Bán and G. Sándor, *Collegium*, 1925, 565.

8 O. Gerngross and G. Sándor, *ibid.*, 1926, 1, 669; 1927, 20, 681.

9 O. Gerngross, N. Bán and G. Sándor, *Zeit. angew. Chem.*, 1926, **39**, 694, 1028.

10 O. Gerngross and G. Sándor, *Collegium*, 1927, 12.

[11] O. Gerngross and H. Hübner, *Collegium*, 1927, 246, 431.
[12] O. Gerngross and H. Hübner, *Ber.*, 1927, **60**, 2094.
[13] O. Gerngross, G. Sándor and K. Tsou, *Collegium*, 1927, 21.
[14] O. Gerngross, *ibid.*, 1929, 512; 1930, 300; *Jour. Int. Soc. Leather Trades Chem.*, 1930, **14**, 325; *Gerber*, 1920, 200; *Cuir Tech.*, 1930, **23**, 32.
[15] O. Gerngross, N. Bán, G. Sándor and K. Tsou, *Der Papier Fabrikant*, 1927, **25**, 49.
[16] V. Kubelka and V. Němec, *Collegium*, 1929, 300; *Jour. Int. Soc. Leather Trades Chem.*, 1929, **13**, 113.
[17] O. Gerngross and K. Tsou, *Der Papier Fabrikant*, 1926, **24**, 497.
[18] O. Gerngross, *Zeit. angew. Chem.*, 1926, **39**, 696; 1928, **41**, 50.
[19] L. Meunier and A. Jamet, *Jour. Int. Soc. Leather Trades Chem.*, 1926, **10**, 212.
[20] H. Kimreuther, E. Schlumberger and W. Nippe, *Der Papier Fabrikant*, 1926, **24**, 106.
[21] W. Leupold, *ibid.*, 1926, **24**, 397.
[22] E. Hägglund and T. Johnson, *Zeit. angew. Chem.*, 1928, **41**, 51.
[23] E. Hägglund and T. Johnson, *ibid.*, 1927, **40**, 1101.
[24] B. Rassow and G. Brandau, *Der Papier Fabrikant*, 1929, **27**, 181, 202, 217.
[25] C. van der Hoeven, *Collegium*, 1930, 414.
[26] L. Pollak and W. Springer, *ibid.*, 1927, 46.
[27] G. Grasser, *Jour. Faculty Agric. Hokkaido Imp. Univ.*, 1930, **23**, 151; *Cuir Tech.*, 1931, **24**, 141.
[28] Meunier and Le Viet, *Cuir Tech.*, 1931, **20**, 312.
[29] M. Cuccodoro, *Boll. Uff. Staz. Sperim. Ind., Pelli*, 1932, **10**, 238.
[30] M. Auerbach, *Collegium*, 1932, 825.
[31] H. Freytag, *ibid.*, 1932, 506.
[32] F. V. Lutati, *Boll. Uff. R. Staz. Sperim. per l'Ind. della Pelli*, 1933, **11**, 436.
[33] J. Desmurs, *Cuir Tech.*, 1932, **25**, 77.
[34] A. Goldberger, *ibid.*, 1932, **25**, 98.
[35] L. Pollak, *Collegium*, 1933, 628.
[36] H. Freytag, *Melliand Textilber.*, 1932, No. 7.
[37] F. V. Lutati, *Jour. Int. Soc. Leather Trades Chem.*, 1933, **17**, 625.
[38] A. Deforge, *Halle aux Cuirs*, 1928, pp. 12 and 83.
[39] A. E. Sampson, *Amer. Dyestuff Reporter*, 1935, **61**, 8.
[40] W. Grassmann and O. Lang, *Collegium*, 1935, 114, 401; *Ind. Chem.*, 1935, **11**, 947.
[41] A. S. Karsten, *Cuir Tech.*, 1935, **24**, 198.
[42] A. S. Karsten, *Ledertech. Rundschau*, 1934, **26**, 93.
[43] A. Borgialli, *Boll. Uff. R. Staz. Sperim. per l'Ind. della Pelli*, 1935, **13**, 245.
[44] G. A. Bravo, *ibid.*, 1935, **13**, 180.
[45] E. R. Theis and E. J. Serfass, *Journ. Amer. Leath. Chem. Ass.*, 1938, **33**, 67.
[46] L. Pollak and A. Patzenhauer, *Gerber*, 1938, **64**, 73.
[47] E. R. Theis and E. J. Serfass, *Jour. Amer. Leather Chem. Assoc.*, 1938, **33**, 67.
[48] A. Noll, *Papier-Fabr.*, 1938, **36**, 41.

CHAPTER X.

LEGAL AND CRIMINOLOGICAL WORK.

THOSE employed in the prevention and detection of crime can often obtain valuable information by the use of filtered ultra-violet radiation. One of the first works dealing with this branch of the subject was a monograph by H. Fischer,[1] which deals with the use of physico-chemical methods in forensic medicine and toxicology and gives special consideration to the fluorescence method; other works on the subject have been published by C. J. van Ledden-Hulsebosch,[2, 3] and by J. Glaister.[62] The simplicity and rapidity with which the examination under the lamp may be made suffice in themselves to give it importance, and this is enhanced by the great sensitiveness of which the method is capable in certain cases. In some instances it may be important that the object under examination shall not be altered in any manner which might show that it had been examined, and here fluorescence analysis has a great advantage over chemical methods. The differences in fluorescence shown by papers, textiles, hairs and fibres, adhesives, various glasses, and other common objects suggest applications of more than passing interest to the police and the forensic chemist.[31, 41, 52] A special apparatus for photographical work in this field is described by L. Bendikson (p. 73).

Customs Examinations.—The customs officials in Germany have used Wood's light for the examination of goods passing through their hands to detect the presence in them of certain dutiable substances. Inspector Lahnstein of the German Customs House gives a list of a number of goods examined in this way.[4]

Raw- and cleaned-rubber are duty-free in Germany, and can be distinguished with ease from other forms, which are dutiable, and crêpe rubber can be differentiated from the low-duty vulcanised rubber. Sequins of gelatine are distinguishable from those made of metal or wood by their bluish fluorescence. Woven straw is free of duty, and, under the lamp, the only portion which fluoresces is the inside surface, which glows with

a bluish light, whereas bleached straw (which carries a duty) fluoresces both inside and out with a bright brown colour. Benzol, which is non-fluorescent, enters the country duty-free, but petrol is dutiable, and relatively small additions of petrol to benzol are detected by means of the bluish fluorescence produced by the former (see also A. Marcan [33]). The American Customs authorities use a similar method to control the distribution of surgical spirit (see p. 136).

In the days of "Bootleg" liquor Goodman suggested a method on these lines in order to ascertain whether the liquor was being made locally, or brought from a distance, or whether it was genuine liquor that had been smuggled into the country.

Boxes of cigarettes are required to bear a revenue stamp, which is sometimes falsified, and such falsification is immediately evident when the stamp is examined under the lamp. Special stamps, in which quinine is incorporated, are now made, so that when placed under the lamp they may be identified by their fluorescence.

H. P. Wunderling [49] cites a case of seizure of "dope," some particles of which melted sharply at 150° and others at 170° C.; in ultra-violet light it was possible to pick these out by hand, and they were shown to be novocaine (violet fluorescence) and stovaine (non-fluorescent), respectively.

In a similar instance [61] a green fluorescence on a piece of wrapping-paper was used to confirm the fact that the paper had been used for smuggling hashish.

Body Marks.—H. Goodman [5] gives a number of instances in which the lamp may be of use to the police. It will be found on observation that a person nearly always wears his wristlet watch on the same wrist and, after some time, a brownish band where the strap of the watch has encircled the wrist is visible under the lamp. Old scars and tattoo marks which have been erased by chemical means, and which do not show in daylight, can also be seen in this way. The same applies to the effects of some skin diseases which leave no trace visible in ordinary light, and dyed hair can be detected at once if the dye is fluorescent. Some firms put traces of highly-fluorescent substances in their products (where the nature of the product permits this), and are thus able to tell rapidly whether a sample is their own article or whether it is an imitation.

A somewhat amusing instance is reported from Chicago, following a £20,000 lawsuit in which two mothers in a large maternity home claimed that their newly born babies had been mixed up. The children are now "sunburnt" with an identification mark after birth, which is visible in ultra-violet light, but not in ordinary daylight.[29, 34]

Documents.—Great assistance is obtained by use of the lamp for the detection of forgeries, erasures and alterations to documents, and many large banks have now installed a source of ultra-violet light for this purpose. D. J. Block [6] and others [67, 68, 70] have patented a "safety" paper impregnated with quinine or its salts, uranium salts, or aesculin, etc. It is claimed that erasures on this paper show up as dark patches when placed under the lamp, and it is suggested that cheques, cash books and other important papers should be treated in this way. H. Goodman [39, 41] has suggested that such identifiable paper should be used for notes paid over as ransom, and that the characteristic fluorescence, especially after addition of sulphuric acid (*e.g.*, to quinine papers), might be of assistance in tracing letters received in such cases. Suitably treated fibres which appear the same as the remainder of the paper in ordinary light, but which produce a silurian effect, in ultra-violet light may also be added in the beater,[66] and such papers would be exceedingly difficult to imitate. Various coloured inks which appear on banknotes, etc., all have characteristic appearances in ultra-violet light, and this is of great assistance in the detection of forgeries. In several recent cases the method was used to show that stamps had been fraudulently removed from an insurance card,[69] or to detect eradicated cancellation marks.[63, 82]

In addition to the suggestions already mentioned,[68, 70] there have been numerous patents [85] within recent years on the subject of identifiable papers containing fluorescent substances (see also under **Inks**). Most of them are complex organic substances having an affinity for cellulose, which, however, are permanent in effect, and do not become visible in ordinary light. They may be added either in the beater or else impregnated on to the paper after it has been made, while some workers (*e.g.*, W. Zänker [86]) prefer to prepare the fluorescent fibres separately and to add them to the paper so as to produce a silurian fluorescent effect.

Bein and H. J. Braun [7] have employed the lamp where

many hundreds of foreign loans and deeds are examined every day, and they conclude that falsification and forgery can be detected much more rapidly than by ordinary scrutiny. Where important documents are concerned in litigation, and such questions as alteration and falsification come into consideration, it is invaluable to have photographic reproductions of the documents taken in ultra-violet light, since if these show up the falsification readily they may be presented to the judge or jury for examination. W. Scheffer and P. Müller,[8] for example, have published photographs of cheques in which chemical erasure and subsequent superimposition of writing can be seen quite plainly.[67, 73] The work of C. Ainsworth Mitchell[40] confirms this conclusion, and one of us (J. G.) has examined every chemical ink-eradicator obtainable, but has been unable to find one which does not leave a mark visible in ultra-violet light. Nevertheless, it seems that after ageing, this mark may disappear (Mitchell,[89] Mansfield[90]) although the rate at which it does so varies considerably from one specimen to another. Mitchell refers to a period of several months and relates the fading to the diffusion of chloride ions from the ink into the paper; the fluorescence of specimens in our possession (J. G.) has, however, hardly altered over a period of five years.

On the other hand, Mitchell found that pencil marks, if not heavy and so long as the surface of the paper is not abraded, can be erased by india-rubber leaving no trace visible in ultra-violet light. He also found (*cf. infra*) that solvents may be used to clean paper, and so render writing visible if viewed in ultra-violet light, and that the different fluorescence colours of various typewriting inks may be used to establish whether typescript has been added to at any time.[63] Further work in this connection has also been done by E. Glimm and H. Schröder,[10] Baker[32] and H. Türkel[11, 56] (see pp. 228 and 278, and Photograph No. 3, facing p. 400).

The investigations of one of us (J. G.[39, 64, 66]) in connection with paper (see also p. 340) have indicated several applications of the method to criminological work. In one instance where the question was raised whether a banknote was a forgery, comparison of the design and paper with those of a genuine note by the usual microscopical methods gave inconclusive results. Examination in ultra-violet light, however, showed that the two papers fluoresced in different shades of pale violet. This indicated that probably

pulps of different bleachability had been used (see p. 336), although this again could not be considered as conclusive evidence, since variations of this order have been known to occur between successive makings from the same mill. The designs, however, showed a very striking difference, being black in one case and pale green in the other, showing that different dyes had been used. It was also shown that in one case the watermark was a forgery (see below). The applications of the method to the dating of documents are dealt with fully elsewhere by J. Grant.[57]

Artificial Watermarks fall into two main categories :—

(*a*) In one type a transparent appearance is produced on the paper by means of a stamp using an oily or waxy medium, and in such cases the design usually fluoresces vividly. In the instance of the banknote forgery examined by J. Grant [39] (see above), the watermark could apparently be removed by sponging with ether, but the place where it had been then showed the design as a vivid fluorescence in ultra-violet light.

(*b*) The other type is produced by "impression," *i.e.*, the paper is impressed with the design by means of a rubber roll before the paper is finally dried. The compression thus renders the paper thinner but denser where the design on the roll touches the paper, whilst with a genuine watermark the fibres are removed from the layer of wet pulp before the paper is really made, so as to produce a true thin place. Although such watermarks show no characteristic differences in ultra-violet light, if the sizing test described by J. Grant [60] (see p. 343) is applied, the genuine watermark will allow penetration through the design more rapidly than through the surrounding paper, and the design will glow vividly for a short time before the rest of the paper. Water diffuses through impression watermarks, however, at the same rate as through the rest of the paper.

Value as Evidence.—So valuable are photographic reproductions that for court work G. Kögel [9] considers that "no photograph, no conviction" should be the rule, although in this connection it should be pointed out that its value so far as convincing a jury is concerned is, at present, necessarily somewhat limited. One cannot do better than quote C. Ainsworth Mitchell,[12, 13] who has stated that "Scientific documentary evidence is, of course, circumstantial in character, and it is a common practice for counsel for the defence in criminal trials to decry all

the circumstantial evidence, notwithstanding the fact that it is often more trustworthy than personal testimony. The rules governing documentary evidence are the same as those governing other expert evidence."

The value of evidence based on scientific investigations and given by an expert witness is, however, being increasingly appreciated by members of the legal profession.[58] Thus, cases are on record where judges have allowed tracings or photographs of handwriting to be shown to the jury. Mitchell (*loc. cit.*) quotes the case of Rex *v.* Podmore (1930) in which the examination of a dirty piece of paper led to the discovery that " Thomas " was an alias of Podmore. The paper was cleaned with solvents, and it was then found that in ultra-violet light much more of the writing was readable than was visible in an ordinary photograph. However, " to avoid any suggestion of the use of the imagination the evidence on this fragment at the trial was restricted to the words and characters which the jury could see for themselves." Photographs of erased cancellation-marks on counterfoils were accepted by the Court as evidence in the " directory fraud " case.[75]

In support of the use of ultra-violet light for such work, it should be pointed out that, as a rule, it is not necessary to damage or touch the document in any way. This objection (sometimes raised by a judge to a chemical examination of valuable documentary evidence) may not, therefore, necessarily apply when fluorescence methods are used.

Inks, Finger-Prints and Stains.—*Inks* in general are discussed on page 278, but Rubner [14] has dealt with the appearance of *invisible inks* in ultra-violet light, and here again photography can record results in such a form that they may be produced for reference at a moment's notice. The ingenuity of prisoners is well known, and saliva, vinegar, salt water, solutions of metallic salts, juices of fruits and vegetables, urine, milk, soap-suds and limewash from the walls, mixed with water, have all been employed as " invisible inks " ; [55] Buhtz [15] mentions also the use of a medical prescription containing bromide as a sympathetic ink. Since all these substances fluoresce more or less, the writing can be seen under the lamp, and correspondence to and from prisoners can be examined rapidly without leaving any evidence of examination as is the case when the paper is heated or treated chemically

(cf. van Ledden-Hulsebosch [16]). The photographic reproduction of a letter in which ordinary ink writing is interspersed with words written in urine [42, 43, 57] is easy to carry out satisfactorily (see Photograph No. 13, facing p. 400). Most red inks fluoresce vividly, and it is frequently possible to use ultra-violet light to read writing which has been transferred to blotting paper by the blotting process, even if the paper has been used a great deal and was originally red in colour.

S. Dutt [76] has shown how fluorescence analysis may aid the identification of the country-made inks used extensively in the rural districts of India. Treatment of the paper on which the writing occurs with ether causes the oil to spread away from the pigment, and after drying a fluorescence typical of the oil used is seen. Dutt gives the colours obtained from various oils, but the method would probably find its greatest use for the comparison of two or more inks. The fluorescence fades in the course of time and eventually disappears, the period of permanence depending on the nature of the oil. Identifiable printing and writing inks which are recognisable by their fluorescence in ultra-violet light either on direct inspection or after treatment with an appropriate reagent, have also been suggested [68, 70, 83, 84] (see also under **Documents**). Fluorene, acridine or auramine are among the substances suggested for the first type, and the reagent used in the second type may be chosen so that its effect on the ink is invisible in visible light; the numerous reactions occurring throughout this book will suggest many possibilities.

Finger-prints.—For the detection and photography of finger-prints the lamp has proved of use, since the impressions normally left by the fingers contain small traces of organic matter, such as oils and fats, and these fluoresce in ultra-violet light. H. L. Brose,[36, 37] in collaboration with the Criminal Investigation Department of Nottingham, has developed this study considerably by the use of zinc sulphide (or anthracene), which is dusted on to the print, the excess being gently blown or brushed off, and the fluorescence in ultra-violet light recorded photographically on Ilford fast plates, or preferably, on Ilford panchromatic plates (exposures 20 and 40 minutes, respectively). In this way records of finger-prints on multi-coloured backgrounds are obtainable with a sharpness hitherto unattainable (Photograph No. 17, facing p. 400). Details of the photographic technique

are dealt with under the appropriate heading (p. 69), but it may be mentioned here that by use of a box with two apertures which opened alternately so as to expose the object to a tungsten arc and to a camera, it was possible to separate the effect of phosphorescence from that of visible light, and the use of a filter was thereby avoided; a blackened conical funnel served to eliminate extraneous light. M. Bornand and G. Bonifazi [54] examine finger-prints after first brushing the finger concerned with nitric acid, and F. F. Lucas [72] has patented a method in which the fingers are treated with diazine fast yellow, after fixing the fatty matters in them; similar methods occur in the patent literature.[80]

Stains on Garments.—Where these have to be examined the lamp can be used with advantage, both for the detection and, in some cases, for the differentiation of the spots. In cases of rape the garments or materials showing spots suspected to be of a seminal nature are placed under the lamp, when the stains due to *semen* stand out clearly with a blue-white fluorescence. F. W. Martin [45] has published some excellent photographs of such stains (see Photograph No. 15, facing p. 400), but he points out that the colour of the fluorescence may be modified by the colour and nature of the fabric. The method is useful to distinguish semen from leucorrhœal stains, which occur in the same positions on clothing and have other points in common with the semen. It is not easy, however, to distinguish stains due to pus, perspiration or nasal mucous from those due to semen. Exposure of stains to ultra-violet light frequently alters their fluorescence,[54] although they retain the power to show a fluorescence for long periods if not irradiated.[56]

The lamp has been used by the Chemical Examiner of Madras [18] to detect semen stains on white or light-coloured cloths, and according to this worker black or blue coloured cloths do not show it. In his opinion ultra-violet light is, however, no test for semen, since many other stains also show up, but it is useful as a sorting test on large pieces of cloth, since it enables one to find which portion of the cloth should be examined in greater detail. R. Heller [19] has also shown that seminal stains may readily be detected in this way, but it is always advisable, when the test is positive, to confirm the presence of spermatozoa by the usual Florence test and by microscopical examination (cf. A. Husson [81]).

According to C. T. Symons [17] stains due to *urine* have a more

yellow fluorescence than those due to semen, and this substance is dealt with further under invisible inks (p. 228) and body fluids (p. 240); the subject of stains has also been studied by F. Wiethold,[46] T. Ito,[47] and Simonin.[48]

A typical example of the use of the lamp for the comparison of *pieces of material* is given in a report of the Government Analyst for Ceylon.[20] A piece of extraneous, torn, common white cloth was found in a house which had been burgled and appeared similar as regards dirt, material, seams, tears, etc., to that found in a suspect's house, although the cloth was so common that this similarity was no proof of identity. Ultra-violet light, however, showed stains (invisible to the naked eye) at the torn edge of one of the pieces and a corresponding stain on the other piece, and on joining the edges together, the stains were shown to be continuous and common to both pieces.

A somewhat similar instance is described by A. Brüning,[38] who was able to trace the path of a bullet through clothing from the fluorescence of the material at the points where it had become scorched. Cases involving eradicated laundry marks which have been rendered visible by ultra-violet light, are also on record,[79] and L. S. Smith [78] has patented a laundry ink which is invisible in ordinary light, but fluoresces in ultra-violet light, even after the article on which it occurs has been washed or cleaned. Provision for reading such inks has been made in a laboratory for laundry research which was opened in 1936.[88]

Bloodstains.—R. Golonsko,[21] M. Wagenaar [22] and R. Heller,[23] in a second paper, have worked on the detection of blood (see also p. 246), and the method evolved is both delicate and specific. Blood in the presence of concentrated sulphuric acid shows a yellow-red fluorescence which changes to a brilliant carmine-red when the solution is made alkaline with ammonia. Concentrated acid is therefore added to the blood, and the mixture is subsequently diluted, and strong ammonia added. The fluorescence is visible when the blood is present to the extent of 0·0002 c.c. in 5 c.c. of water, or when 0·5 c.c. of a 0·1 per cent. solution of blood is treated with acid.

In his subsequent work Wagenaar [44] has used a fluorescence microscope and micro-spectrometer, a few threads of the material being treated with a minimum quantity of strong sulphuric acid on a microscope slide, and a cover-slip applied. If a drop of

acetone is then drawn under the cover, extraction of the non-fluorescent hæmatin, and local heating to convert it into hæmato-porphyrin occur, and the fluorescence-colour or principal spectrum lines of the latter (598 and 550 $m\mu$) may be observed; addition of ammonia produces the violet colour (lines at 615, 574, 544 and 509 $m\mu$). Another method [63] is to remove the iron with concentrated sulphuric acid, the excess being partly blotted off and the remainder neutralised with ammonia, when a scarlet fluorescence appears; in this way it has been possible to distinguish rust stains from bloodstains on a knife. R. M. Mayer,[59] who has made a study of bloodstains on leaves exposed to the weather, was able to convert them into proto-porphyrin by the method of Papendieck and Bonath, and to remove the iron by means of hydrazine; the porphyrin could then be extracted in 3 per cent. hydrochloric acid, the chlorophyll being unaffected.

Further work on the detection of blood and other stains has been carried out by H. Gilbert.[24] He recommends the substitution of the ocular of an epimicroscope by a micro- or ordinary spectroscope in order to eliminate, or greatly reduce, the personal factor in the examination of the stains in filtered ultra-violet light. With old stains it may be necessary to add a little hydrogen peroxide in order to obtain the characteristic lines of oxyhæmoglobin. Photographs of bloodstains on cloth by Dr. F. W. Martin are shown in Photograph No. 14, facing p. 400.

Seals, Adhesives, etc.—The various adhesives and other sealing substances show fluorescences under the lamp which differ widely in colour, and this facilitates detection of the opening and subsequent resealing of letters and parcels. *Dextrins* fluoresce, but *gum-arabic* is practically devoid of fluorescence, whilst the gum or mucilage used for postage stamps shows a strong bluish fluorescence similar to that from starch. *Glue* has a strong fluorescence both in the solid form and in solution, and vegetable glues made from soya bean meal can be differentiated from casein glues, since the former appear golden-yellow, and the latter bluish-white.

Sealing-wax, which has been partly burnt by exposure to the bare flame used to soften it, differs in fluorescence from wax which has been melted in a cup or thimble; recently-melted wax differs from that which has not been melted for some time,

and it is possible that natural ageing may also make a difference. These are facts which should be borne in mind when sealing-wax is in question, but in general, distinct differences are shown by various waxes which appear identical in daylight.

When a seal has been tampered with the tiny particles of the substance used to make the original seal can often be detected by variations in colour or intensity of the fluorescence under the lamp. An interesting case is quoted by Symons [17] in which gems to the value of £50,000 were involved. They were insured for this sum and were then sent by post to another country, where, on arrival, the package was found to be empty. The parcel had been sealed in six places, and it was found, by means of the lamp, that one of these seals had been broken, and skilfully made good. In another instance [91] in which Rs. 25,000 had been removed from a sealed parcel, it was possible to show that the seals had been made good with the same wax as used originally, and this was of great assistance in tracking the thief. In connection with this case it was found that the best results are obtained by crushing a little of the wax in alcohol, centrifuging, and examining the clear liquid in a silica capillary tube.

Examination of Clues.—Clues, such as matches, pieces of paper and cloth, found on the scene of a crime may be compared in ultra-violet light with objects found on the suspected person, and the examination of spots or stains on textiles, *e.g.*, carpets and clothes, has been carried out by van Ledden-Hulsebosch [2, 3] and by C. Goroncy.[25] In one case it was desired to know if a motor-car had been involved in a certain accident which occurred on a road on the surface of which were many small pieces of glass and rubbish. The headlights of the suspected car had been smashed, and on examination of the fluorescence of the pieces of glass remaining in the lamp and of the glass from the road it was possible to show the presence of the former among the latter. This was considered good presumptive evidence as to the whereabouts of the car at the time of the accident. In a more recent instance [71] of a similar nature fragments of glass in an injured man's coat were identified with glass from a rubber-mounted windscreen. The identification of glass is also discussed on page 113.

A similar instance is described by H. Goodman [30, 35] in connection with a case in which claims were made regarding

the permeability of a "health window glass" to the beneficial rays of ultra-violet light; he was able to obtain a fluorescence with salicylic acid (due to rays of wave-length below 3200 Å.), using a certain filter, but not with ordinary window glass or with the glass in question. N. A. Marris [53] describes a "smash-and-grab" case in New Zealand in which he was able to identify the glass splinters in an attache case alleged to be used for the purpose, with those from the broken shop-window; fluorescence methods were used in conjunction with determinations of refractive index and specific gravity.

H. Klauer [50] was able to use the lamp to obtain evidence that led to a conviction in a case of murder, which on first consideration had all the semblance of a suicide. A woman was found strangled, and it was alleged that this was done with a rope, one end of which was drawn over a door. Portions of this end, however, differed in fluorescence from the rope actually used, and characteristic rust-coloured marks were also apparent on the door-handle but were absent from the rope.

Sir B. Spilsbury [65] has cited a case in which the difference in fluorescence between a dressing worn on a wound by a man accused of murder and a piece found on the scene of the crime was used as evidence of the innocence of the accused. The ladder in the Lindbergh case was also examined in ultra-violet light, and a case is on record [77] in which a suspected thief was trapped by applying anthracene powder to some money before the theft, and showing by the fluorescence method that the anthracene was present on his hands and clothes after the theft.

Drugs, etc., in Body Fluids.—The French group of workers [26-28] has obtained some results which, however, at the moment are only of academic interest, although with further extension they might be applied to forensic work. It was found that certain drugs taken into the system by way of the mouth were expelled in the milk, saliva and urine, and could be detected and even estimated by means of the lamp (*cf.* pp. 254 *et seq.*). Thus a dose of basic quinine sulphate containing 0·365 grm. of quinine was detectable in the urine for nine days and in the saliva for five days after. The quinine base recovered in the urine was estimated at 65 per cent., and this quantity was checked by means of a polarimeter, which gave slightly higher results; the amount in the saliva was too small to be obtained quantitatively. Human

milk, if made slightly acid by the addition of sulphuric acid, showed the fluorescence reaction for two days after administration of the quinine. In a fatal case [73] of celandine poisoning in which the chemical and botanical examinations gave negative results, ultra-violet light produced a marked yellow fluorescence in the intestines, which was similar to that produced by the juice of celandine.

The same method enables the elimination of hydrastine in the urine to be followed, and 3 per cent. of the alkaloid was easily detected over a period of five days. Another experiment, in which aspirin was used, showed that salicylic acid could be detected in the milk by the violet fluorescence produced when the milk was made alkaline (*cf.* p. 166). Radley has found that a heavy cold, or recent consumption of eggs, induces an intense blue in urine itself, and this may possibly be due to the passage of traces of phosphates or of albumen. Further mention of this type of work is made in Chapter XI.

The advantage of such methods is that only small quantities of the fluid are necessary for examination (only 2·5 c.c. were required in many of the experiments mentioned above), and further investigations on these lines for the detection of other drugs excreted by the body fluids should be of great interest to the expert witness, in spite of the statement of F. W. Martin [51] who considers that in poisoning cases the use of the method is limited to a relatively few instances, *e.g.*, with calomel, where the fluorescence is particularly outstanding.

REFERENCES.

[1] H. Fischer, " Die physikalische Chemie in der gerichtlichen Medizin und in der Toxikologie, mit spezieller Berücksichtigung der Spektrographie und der Fluorescenzmethoden " (Zürich: A. Rudolf), 1925.

[2] C. J. van Ledden-Hulsebosch, " Moderne Dokumentenuntersuchung " (Amsterdam), 1923.

[3] C. J. van Ledden-Hulsebosch, *Arch. f. Kriminologie,* 1926, 78, 1.

[4] Lahnstein, *Zollrundschau,* 1927, no. 18.

[5] H. Goodman, *Police Jour.*, 1931, 4, 225.

[6] D. J. Block, *U.S.P.*, 1,771,612, 29/7/30.

[7] Bein and H. J. Braun, *Chem. Ztg.*, 1928, **52**, 319.

[8] W. Scheffer and P. Müller, *Tech. Rundschau. Wochenschr. d. Berliner Tageblattes,* 1927, **33**, no. 40.

[9] G. Kögel, " Die unsichtbaren Strahlen im Dienste der Kriminalistik " (Graz: Ulr. Moser), 1928.

[10] E. Glimm and H. Schröder, *Kriminologie,* 1928.

[11] H. Türkel, *Intern. Oeffentl. Sicherheit,* 1926, no. 15.

[12] C. Ainsworth Mitchell, *Analyst*, 1932, **57**, 144.
[13] C. Ainsworth Mitchell, "The Scientific Detective and Expert Witness" (Cambridge: Heffer & Son, Ltd.), 1931.
[14] Rubner, *Arch. f. Kriminologie*, 1926, **79**, 4.
[15] G. Buhtz, *Deut. Poliz.-Archiv.*, 1926, **5**, 314.
[16] C. J. van Ledden-Hulsebosch, *Arch. f. Kriminologie*, 1927, **80**, No. 4.
[17] C. T. Symons, *Police Jour.*, 1930, **3**, 235.
[18] Annual Report of Chem. Examiner of Madras, 1930; *Analyst*, 1931, **56**, 664.
[19] R. Heller, *Vjschr. gerichtl. Med.*, 1915, **50**, 37.
[20] Annual Report Gov. Analyst, Ceylon, 1930; *Analyst*, 1931, **56**, 664.
[21] R. Golonsko, *Inaug. Diss.*, Zürich, 1916.
[22] M. Wagenaar, *Pharm. Weekblad*, 1930, **67**, 415.
[23] R. Heller, *Vietelj. ger. Med. u. öffentl. Sanitätswesen*, 1916, **51**, 219.
[24] H. Gilbert, *Ann. Méd. légale*, 1929, **9**, 541.
[25] C. Goroncy, *Kriminal. Monats.*, 1927, no. 9.
[26] E. Bayle and Fabre, *Compt. rend.*, 1925, **180**, 605.
[27] E. Bayle, R. Fabre and H. George, *Ann. Méd. légale*, 1925, **5**, 11.
[28] R. Fabre, *Bull. Soc. Chim. Biol.*, 1925, **7**, 1024.
[29] Anon., *Daily Telegraph*, July, 1931.
[30] H. Goodman, *Amer. Jour. Phys. Therapy*, 1930, **7**, 7; 1932, **9**, 147.
[31] E. W. Stein, *Sci. American*, Oct., 1932.
[32] G. W. Baker, *Analyst*, 1933, **58**, 159.
[33] A. Marcan, "The Chemist in the Far East" (London: The Institute of Chemistry), 1933.
[34] H. Goodman, *Amer. Jour. Phys. Therapy*, 1928, **5**, 80.
[35] H. Goodman, *ibid.*, 1930, **7**, 7.
[36] H. L. Brose and C. G. Winson, *Nature*, 1933, **132**, 208.
[37] H. L. Brose, *Analyst*, 1934, **59**, 25.
[38] A. Brüning, *Arch. f. Kriminologie*, 1931, **88**, 197.
[39] J. Grant, *Analyst*, 1933, **58**, 603.
[40] C. Ainsworth Mitchell, *ibid.*, 1933, **58**, 532.
[41] H. Goodman, *Chicago Police Mag.*, 1933, Jan. 1934.
[42] H. Goodman, *Police Jour.*, 1931, **4**, 430.
[43] F. W. Martin and K. Beöthy, *Lancet*, 1934, **226**, 1006.
[44] M. Wagenaar, *Pharm. Weekblad*, 1934, **71**, 478.
[45] F. W. Martin, *Brit. Med. Jour.*, 1934, No. 3809, p. 37.
[46] F. Wiethold, *Deut. Zeit. Ges. Gericht. Med.*, 1926, **7**, 600.
[47] T. Ito, *ibid.*, 1927, **9**, 726.
[48] Simonin, *Ann. Méd. légale*, 1929, **9**, 60.
[49] H. P. Wunderling, *Document Investigation*, 1933, **1**, 13.
[50] H. Klauer, *Deut. Zeit. Ges. Gericht. Med.*, 1933, **20**, 375.
[51] F. W. Martin, *Analyst*, 1934, **59**, 531; *Police Jour.*, 1934, **7**, 4.
[52] W. M. Else and J. M. Garrow, "The Detection of Crime" (London: *Police Jour.*, 1934).
[53] N. A. Marris, *Analyst*, 1934, **59**, 686.
[54] M. Bornand and G. Bonifazi, *Pharm. Acta Helv.*, 1933, **8**, 107.
[55] B. Langen, *Med. Klin.*, 1930, No. 36.
[56] S. Türkel and J. Daimer, "Geheimtinten" (Graz: Moser, 1931).
[57] R. Mellet and A. R. Bischoff, *Compt. rend.*, 1925, **181**, 868.
[58] A. Brüning, *Arch. Krim.*, 1931, **88**, 197.
[59] R. M. Mayer, *Deut. Zeit. Ges. Gericht. Med.*, 1933, **20**, 577.
[60] J. Grant, *Analyst*, 1934, **59**, 749.

[61] Ann. Rept. Gov. Analyst, Palestine, 1933; through *Analyst*, 1934, **59**, 752.
[62] J. Glaister, *Practitioner*, July, 1934.
[63] C. A. Mitchell, *Medico-Legal and Crim. Rev.*, 1935, **3**, 3.
[64] J. Grant, *ibid.*, 1935, **3**, 14.
[65] B. Spilsbury, *ibid.*, 1935, **3**, 19.
[66] J. Grant, *Proc. Tech. Sec. Paper Makers' Assoc.*, 1935, **16**, 97; *Paper Trade Rev.*, 1935, **103**, 1463.
[67] J. B. Meyer, "Die Sicherungstechnik der Wertpapiere" (Zurich: Paco), 1935.
[68] J. Waterlow & Sons, E.P. 292,393, 16/9/27; 417,488, 5/10/34.
[69] *Luton News and Bedfordshire Advertiser*, 16/5/35.
[70] I.G. Farbenind., E.P. 390,188.
[71] Anon, *Daily Telegraph*, 1/3/38.
[72] F. F. Lucas, U.S.P. 2,066,535, 5/1/37.
[73] M. Jamieson, *Analyst*, 1937, **62**, 743.
[74] B. Behrens, *Deut. Zeit. Ges. Ger. Med.*, 1937, **29**, 171.
[75] Anon, *Daily Telegraph*, 17/11/36.
[76] S. Dutt, *Document Investigation*, 1936, **4**, 3.
[77] Anon, *Analyst*, 1938, **63**, 502.
[78] L. S. Smith, U.S.P. 2,056,809, 6/10/36.
[79] Anon, *Chem. and Ind.*, 1936, **55**, 1032.
[80] Electrical Research Products, E.P. 35,220, 1936; 473,043, 1937.
[81] A. Husson, *Rév. Intern. Crimalist.*, 1934, **6**, 407.
[82] Anon, *Evening Standard*, 24/9/35.
[83] F. M. Sell, U.S.P. 2,073,381, 9/3/37.
[84] R. Roley, *Amer. Ink Maker*, 1938, **16**, 18.
[85] Imperial Chemical Industries Ltd., C. Paine and J. A. Radley, E.P., 442,530, 1934.
[86] W. Zänker, F.P. 806,805, 26/12/37.
[87] J. Grant, "Books and Documents. Dating, Permanence and Preservation" (London: Grafton & Co.), 1939; *Librarian*, 1939, **28**, 146.
[88] Anon, *Chem. and Ind.*, 1936, **14**, 1032.
[89] C. A. Mitchell, *J. Inst. Chem.*, 1938, 473.
[90] W. R. Mansfield, *Medico-Legal and Crim. Rev.*, 1937, **7**, 29.
[91] Govt. of Madras, *Ann. Rept. Chem. Examiner*, 1937; *Analyst*, 1939, **64**, 120.

ADDITIONAL REFERENCES.

H. P. Wood, U.S.P. 2,016,474, 8/10/35 (Apparatus for examining cheques in ultra-violet light).

A. Lucas, "Forensic Chemistry and Scientific Criminal Investigation" (London: Arnold), 1935.

R. B. Haselden, "Scientific Aids for the Study of Manuscripts" (Oxford, University Press), 1935. (See also Chapter XIII.)

W. R. Mansfield, *Phot. Jour.*, 1935, **75**, 169 (Documents and writing).

C. A. Mitchell, "Documents and their Scientific Investigation" (London: Griffin), 1935.

C. A. Mitchell, "Inks" (London: Griffin), 1937.

C. A. Mitchell, *J. Inst. Chem.*, 1937, 59 (General).

R. Roley, *Amer. Ink Maker*, 1938, **16**, 18 (Fluorescent and invisible inks).

M. Déribéré, *Papeterie*, 1936, **58**, 1022; 1937, **59**, 369 (Fluorescent paper).

S. Smith and J. Glaister, "Recent Advances in Forensic Medicine," (London: Churchill), 1939.

CHAPTER XI.

MEDICAL AND BIOLOGICAL SCIENCE.

Zoology and Animal Tissues.

WHAT is probably the first mention of the fluorescence of animal tissues was made by Brewster,[1] and the next by Helmholtz [2] who noted the fluorescence of the eye (see pp. 73 and 241). The next work on the subject was due to Köhler,[3] and, in 1911, Hess [4, 5] examined the eyes of insects, crabs, and the lucidum of mammals. Goldstein [6] mentions the fluorescence of hair, nails, skin and eyes, while Kowalski [7] also noted that the skin, muscles and certain bacterial growths fluoresce. In the same year H. Stübel [8] used the lamp to examine animal tissue taken from vineyard slugs, fresh-water crabs, frogs and dogs, and F. Querner [77] records the fluorescence of the livers of vertebrates in radiation of wave-lengths of 3,000 to 4,000 Å.

Stübel emphasises the importance of the modification of the fluorescence by the presence of *pigments* and of *hæmoglobin* or its derivatives, and observed that the greater the quantity of these present, the more the fluorescence of a tissue deviates from white. Thus, whilst a specimen deprived of its hæmoglobin fluoresces with a bluish-white colour, the same specimen containing hæmoglobin has a yellow or brown fluorescence. The muscles of certain vertebrates thus probably owe the colour of their fluorescence to their hæmoglobin content.

Skin deficient in pigment is more fluorescent than that which is highly pigmented, and white hairs stand out among brown or black hairs very distinctly, as they have a strong bluish-white fluorescence. It is interesting to note that blonde hair may fluoresce with a variety of colours (blue, silver, white, yellow, etc.), but "artificial blondes" do not; and that skins of white races fluoresce but those of black races do not. Erythema patches on white persons appear dark, but are white on coloured subjects.

Goodman [86-89] considers that it is possible in this way to detect and estimate the amount of radium or X-ray overdosage. In general, the more compact the tissue the stronger is its fluorescence; thus tissue has a stronger fluorescence when dried than when freshly prepared, tendons and sinews fluoresce more than muscles, and bones have a still brighter fluorescence. It is therefore important that all specimens examined in the moist condition should be freshly prepared, as decomposition or bacterial action may alter or even destroy the colour of the fluorescence.

Bommer [83] has photographed abnormalities of the skin, a 5-minute exposure being required when a filter for the ultra-violet light (see p. 77) is used, but otherwise 1 to 4 seconds. A. Furniss [84] extracted quinoidine from the skin with boiling hydrochloric acid (the Bence-Jones method), and found that it was strongly fluorescent. Suprarenal glands appear a deep orange-red, liver an intense yellow-green, and the cortex of the brain yellow. The mineral substances responsible for the fluorescence of the skin of the frog, *Rana esculenta*, are principally manganese and zinc.[183] The scales of certain fresh-water fish contain flavins which frequently have a blue fluorescence, although treatment (*e.g.*, with acetic acid) is sometimes necessary to develop it.[189]

J. Turchini [9] has investigated the fluorescence of the tentacles of *Anemonia sulcata* (yellowish-green), *Heliactis bellis*, *Calliact effeeta* and *Actinia equina*. A beautiful fluorescence is shown by the "red bands" of *Echinus acutus*. A yellow fluorescence is obtained from the *Antedon* and violet fluorescence from the chalk lamallæ of the star fish and *Holothurian*. Protozoa fluoresce and can often be identified by their appearance under the lamp, and it is possible also to recognise the food vacuoles and other organs of the paramæcium. In conjunction with H. Havant,[10] Turchini examined the parente between the vacuole cells and the renal vesicules of the *Ascidia mentula*, Müll. The envelope around the renal region shows under the microscope a lilac, pearly fluorescence, whilst the testa of the back, the cell vacuoles, blood and renal vesicules had a lilac fluorescence. A similar but less intense fluorescence is shown by the water in which they live. With O. Duboscq,[11] J. Turchini has also investigated the orange fluorescence of the tergum pigment areas of the *Squillamantis Rond.*

E. Merker[12] notes that the eyes of night-flying moths (*e.g.*, *Bombycidæ*, *Sphingidæ* and *Noctuidæ*) fluoresce, and J. C. Moltram and E. A. Cockayne[13] note that a few of the Lepidoptera, usually those white or yellow in colour, fluoresce. The most brilliant fluorescence is shown by the *Geometrid* moths of the genus *Ourapteryx*. Fluorescent specimens occur in the other Geometrid genera, in *Uraniidæ* and *Hespialidæ* and in butterflies of the genus *Troides*. By dissolving the wings of *Poisthograptis* and *Troides* in glacial acetic acid a yellow solution is obtained, which has a green fluorescence in daylight. W. S. Andrews[14] mentions the beautiful blue fluorescence given by an aqueous extract of the fire-fly *Photinus pyralis*, in ultra-violet light.

P. Wels[15] has examined the fluorescence of frog muscles and the germ cells of the clam (*Mytilus edulis*), and he showed that this fluorescence is intensified by prolonged exposure to the rays of the lamp, especially in the presence of oxygen. The spontaneous movements of the sperm indicate that the occurrence of fluorescence is possible in the living cell.

E. Newton Harvey[16-18] has studied the bioluminescence and fluorescence of *Ctenophores*, and the fluorescence of the crustacean *Cypridina hilgendorfie* and other plant and animal tissues, and quantitative data are given by Peacock[19] concerning the reaction to ultra-violet light of irradiated tissue.

Tissues[79] containing fat fluoresce strongly, and although urea and uric acid themselves do not fluoresce, an intense bluish-white fluorescence (see p. 230) is given by *urine* under certain conditions. Dried urine[80] on textiles fluoresces with a yellowish-white colour, and semen (see p. 254) shows up with a bright white fluorescence. Gallstones show various colours, from red to violet, and pure cholesterol stones fluoresce with a yellow-green colour (*cf.* p. 230).

P. Ellinger and W. Koschara[90] have used " Intravital Microscopy " (see Fluorescence Microscopy, p. 78) to follow the isolation of animal pigments, such as lyochrome (see below), the green fluorescence serving as a control test ; this is destroyed by light, and is changed to violet by the action of acids and alkalis.

An interesting case is reported by J. B. Bateman[91] who cites correspondence in *The Times* and *The Observer* concerning a woman in Italy who luminesces when asleep and who is psycholo-

gically abnormal (religious and hysterical). The phenomenon is variously attributed to manifestations of holiness, bacterial infection or blood radiations causing luminosity of the skin.[171] Bateman[170] rejects mitogenic radiation as an explanation, for if it does exist it must be too feeble to be apparent in this way. The colour reactions and the fluorescence of the proteins and their derivatives have been studied by A. Roche.[160]

Animal Organs.

The lens of the *eye*, the *blood* and the *skin* act as protective agents against ultra-violet light, and absorb it to a great extent degrading it to visible light. When the eye is examined in filtered ultra-violet light, the sclerotic layer and cornea fluoresce bright blue, but the pupil remains dark, and the white of the eyes of some animals often shows a weak yellowish fluorescent network. The harder parts of the skin show up more than the softer portions, and all scars, pigment spots and traces of skin diseases, such as subcutaneous eczema, which alter the structure of the skin, may be clearly seen. The examination of the effects of dermatitis and of pigment anomalies are therefore easily carried out. H. von Euler and E. Adler[158, 179] obtained a substance with a blue fluorescence from the eyes of fish of certain species which resembled a flavin although it showed different colour changes when the pH value was altered. Saidman and Dufestel (see p. 73) have attempted to use the limit of visibility of the eye in the ultra-violet as a means of estimating the age of the crystalline lens. As may be imagined the fluorescence of the various parts of the eye is a valuable aid to ocular surgery, and M. Van Lint[178] describes its use to render the vitreous visible, especially during the intracapsular extraction of a cataract, or extraction of the luxated or sub-luxated vitreous. In the extraction of a soft cataract or of a transparent vitreous in myopia, aspiration of the vitreous is a frequent procedure, and is facilitated by ultra-violet light.

Scurf fluoresces with a very strong white colour, and psoriasis scales, which show a chalky white fluorescence, can easily be distinguished from the silvery white fluorescence of hyperkeratose. Small abscesses such as acne have a light yellowish-green colour. S. G. Newman[159] was able to detect a single hair

infected with ringworm in a normal head; the method, however, gives a negative diagnosis with "black dot" ringworm, and is ineffective in the presence of ointment. It has been stated that the fluorescence of hair is a guide to the race of the individual but attempts to verify this for hairs from Europeans, Chinese, Kaffirs, American negroes, Syrians, Red Indians, Maoris and half-castes of these races, were a failure [164]; the fluorescence colours merely correspond with those observed in visible light.

Margarot and Devèze,[20] and H. Goodman,[21] and also P. Keller [22] have investigated the fluorescence of *microspores*. They find that hair permeated with microspores or trichophyton fluoresces with a bright green colour, and this method is now used in a number of hospitals and school clinics for the detection of ringworm infection. H. Fuhs [23] is, however, rather sceptical as to the efficacy of the method (*cf.* Lewin and others [24-28]), although A. M. Davidson, S. A. Boyd and C. P. Haltalin [175] have described a simple and convenient apparatus for the purpose. A. Furniss,[176] too, has developed the method considerably, and he finds it of particular value in cases of doubt for convincing parents that the disease is present; ointments and vaseline which often have a fluorescence similar to that due to the disease, should however, first be removed. The method is of greatest use in the diagnosis of ringworm of the scalp (*Tinea tonsurans*), but it can also be used for ringworm of the hairless skin (*T. circinata*) when this is due to microspores.

A. Davidson and P. H. Gregory [101] have examined the fluorescence of *Microsporon audouini*, *M. felinium*, *Trichophyton gypseum*, *T. violaceum*, *T. album* and *Achorion schoendeine*. Of these, the species belonging to the genera *Microsporon* and *Achorion* showed an intense green fluorescence in the substance of the infected hair; this was readily soluble in hot water or in a 7 per cent. solution of potassium hydroxide, and these workers believe that its absence or presence is pathognomonic. J. Kinnear [102] and also H. McCormich Mitchell [103] confirm the characteristic nature of the fluorescence of *M. audouini*. The latter worker also uses the lamp for the detection of *Tinæ Capitis*, and notes that hairs infected with *Trichophyton Megalosporon* have a white luminous fluorescence. Kinnear states he has never found a fluorescent hair which on microscopical examination proved uninfected, or an infected head on which the hair did not fluoresce. A. G.

Millot Severn,[104] however, considers that negative results in this test are not necessarily conclusive, as he found three positive cases which showed no fluorescence; the infection appeared to be a variety of *Endothrix Trichophyton*. For further work the reader is referred to the references.[105-108]

The *teeth* of a normal person fluoresce intensely white, and show the strongest fluorescence of any external organ, but in an old person, or when the teeth are defective, the fluorescence is often reddish; A. A. Hymans van den Bergh and Hyman [29] attribute this red fluorescence to the porphyrin present, and Tiede and Chromse,[161] who were able to reproduce the fluorescence of natural teeth by heating apatite preparations with proteins, consider the latter to be responsible. It seems in fact, unlikely that the type of phosphor produced by firing a salt with a small amount of foreign metal as activator (see p. 54) would account entirely for the fluorescence of teeth. Experiments on these lines by W. H. Byler [180] suggest in fact, that a complex made up of calcium phosphate having an apatite structure and containing about 1 per cent. of organic matter may be responsible, and that the physical (*e.g.*, crystalline) condition is of importance as well as the chemical composition.

The mucous membrane of the tongue shows a greyish to yellowish-white fluorescence, and towards the back of the tongue one can often see a reddish or orange colour. S. Hoshijima [85] has examined the fluorescence spectra of bones, teeth (calcined and otherwise), cartilage, nails and tendons, and finds that the spectra of ashed bones and a mixture of calcite and hepatite are identical. Bones and cartilage decalcified with nitric acid were not fluorescent. However, burnt bones from a tomb 300 years old, which were examined by van Ledden-Hulsebosch (see p. 284), did not fluoresce. Small amounts of manganese and zinc have been held to be responsible for the fluorescence of the ash from the bony tissue of frogs.[165]

S. Bommer [30, 31] has applied the lamp to the study of the skin and to the differentiation of the inner organs, *e.g.*, nerves and blood-vessels, which are traceable by the yellowish-white and bluish-white fluorescence, respectively. The fluorescence of *cartilage* varies with age, being bluish-white in adults and pure intense blue in children less than one year old. *Bones* have a white or yellowish-white fluorescence, whilst the skeleton musculature

does not fluoresce but appears as a rule black to brown, although in puppies it is often a greenish-yellow colour.[79] C. J. Sutro and M. S. Burman [195] have used the method to render visible the biliary system.

Striking fluorescence colours are often shown by the various *glands* of the body, especially by those of animals and insects. The thyroid gland, for example, has a reddish-grey fluorescence, the sclerotic glands a characteristic colour, and the pancreas a weak brownish-yellow fluorescence. Liver varies in colour from brownish-yellow to intense yellowish-green, and a cross-section of the testicles glows dark greyish-red.

It is important to point out here that the presence or absence of fluorescence, and its colour, depends on the organ rather than on the animal from which it is obtained. Furniss states that if a tissue is fluorescent then the extracts of its ash will also be fluorescent, and vice versa.

Various fluorescence colours are given by different portions of the *brain*. Tissue appears yellow coloured, the white matter of the brain has a white fluorescence with a strong red tinge, the ganglions are more greyish-yellow and sometimes reddish-yellow, and the cerebellum is greyish-yellow also. An interesting observation has been made by C. J. van Ledden-Hulsebosch, who has examined the brains of a number of inmates of an asylum and considers that it is possible to differentiate between perception and emission centres; he has also noted and classified other peculiarities, but his work requires confirmation.

The fluorescence microscopy of *animal tissues* has been studied by M. Haitinger and H. Hamperl [92] (see Fluorescence Microscopy, p. 78) who stain the tissues with various fluorescent compounds. Some of their results are given in Table 13A, and others are illustrated in Photograph No. 21, facing p. 400.

" E " indicates that the primary fluorescence was so intense as to mask the secondary fluorescence. F. Querner and K. Sturm [155] have applied similar methods to the histological studies of the liver cells. Further workers who have studied the fluorescence of living tissues are P. Ellinger and A. Hirt,[93-95] W. Hartoch,[96] A. Grabner,[97] E. Baum [98] and others.[99]

The spectra of the fluorescence of some *carcinogenic substances* [193] have been examined by E. L. Kennaway and I. Hieger [32] who noted that these spectra are often the same in type, and they

TABLE 13A.

Substance.	Fluorescence in Neutral Solution.	Cell Nucleus.	Mucus.	Medullary Layer.	Elastic Fibre.	Collagen Fibre.	Muscle.	Fat Cells.	Concentration 1 part in
Aurophosphine . .	Strong green	Yellow-green	Green to brown	E	E	E	Bright green	Blue	50,000
Berberine sulphate . .	Weak yellow	Yellow	—	—	E	E			500,000
Rosol red .	Orange-red	Yellow-red	—	—	Red	E	Red	Opaque yellow-green	50,000
Geranine-G. .	Weak violet	—	—	Rose	White	Red	Red	Blue	10,000
Thiazol yellow-G. .	Violet	—	—	—	Yellow-green	Yellow	Blue		100,000
Thioflavin-S.	Strong blue	Bright blue	Blue	Blue	Bright yellow	Yellow	Yellow-green	Dark blue	1,000,000
Chelidonium (extract) .	Yellow	Golden-yellow	—	—	E	E	—	Turquoise blue	—
Rhubarb (extract) .	Green	Pale yellow to yellow-green	—	—	E	Green	Bright green	Golden-yellow	—

suggest the use of the method for the examination of substances suspected to be carcinogenic in nature. The effect on mice of such substances is, of course, necessary for conclusive proof, but important results may follow if the method is confirmed (see "Fluorescence Serology," p. 249). The work of G. M. Blech [166] has shown conclusively that malignant tissues have a fluorescence which is different from that of normal tissues, and that although the usefulness of the method is limited when a tumour is hidden under the skin, it provides an early if tentative warning in other cases.

J. W. Cook and his co-workers [109-112] have isolated (by means of the picrates) the carcinogenic constituents of coal tar and pitch, and have obtained an active hydrocarbon 1.2–benzpyrene. Since this substance has a typical fluorescence spectrum with bands at 4000, 4180, and 4400 Å., it is considered that the fluorescence test can predict carcinogenic properties, although certain of the cancer-producing hydrocarbons (*e.g.* 1.2.5.6–dibenzanthracene) have spectra which differ from those of active pitch fractions. I. Hieger [113] (see also E. L. Kennaway [115-117]) has obtained carcinogenic compounds by the action of aluminium trichloride on tetralin, and these have spectra similar to that of 9.10–dibenzyl–1.2.5.6–dibenzanthracene. Cook [167] subsequently used the fluorescence spectrum method as a means of control in the fractional distillation of 2 tons of coal-tar pitch, and so was able to select fractions which after extraction with the solvents and crystallisation as picrates, yielded a few grams of a substance which was identified with synthetic 1.2–benzpyrene. In this same connection, C. Sannié [168] believes that the fluorescence spectrum characteristic of purified 1.2–benzpyrene would be unrecognisable in complex mixtures, and although this is denied by W. V. Mayneord,[169] the method was used by H. Druckrey [198] to follow the solubility of benzpyrene in body fluids.

A number of workers have applied the methods of fluorescence analysis to *silk-worm culture*. The cocoon has been studied by L. Lombardi,[33] the eggs by A. Tonon,[34] and the larvæ by S. Beer.[35] A. Policard and A. Paillot [36] have shown that the silk-worm while spinning the cocoon has a yellowish fluorescence in some parts of the body, which was shown to be due to the blood. The blood of the worms of all types and breeds shows a bright yellow fluorescence if removed from the body by means of a

pipette. M. T. Ertrogroul [37] has noted that the progress of the attack of silk-worms by "grasserie" disease can be followed under the lamp, from the spreading of the layer of bright yellow fluorescent liquid which makes its appearance with the disease. Ertrogroul, who innoculated healthy worms with the blood of an infected worm and followed the various phases of the disease, showed that infected blood on mulberry leaves fed to healthy worms produced infection. A. Segitz [38] therefore suggests that the method might well be applied by the silk-worm breeder to the elimination of unhealthy worms. In the healthy worm the yellow fluorescence noted by Policard and Paillot appears to be independent of the quality of the food, since it appears only at certain stages of the larval life and disappears at the end of the pupal stage. If owing to bad feeding or other causes the silk-worm is unhealthy, then the fluorescence cannot be seen. J. Kodahira [39] has also investigated the fluorescence of the humour of the silk-worm (see also p. 365), and his conclusions have been confirmed by S. Beer.[118-120] M. Oku [181] has found that the colouring matter of certain cocoons contains a constituent which has a violet fluorescence, and that this may be sericin and or fibroin.

J. Turchini and J. Millot [40] have investigated the fluorescence of the glands and certain elements in the blood of *spiders*, by the same methods used for silk-worms (see *supra*). All genera of spiders discharge a thread which gives a bluish fluorescence, and the larger glands of the *Pholziden* and the ampule of the ampulliform glands in all spiders have an intense turquoise fluorescence. The brownish tubular glands of the female common garden spider fluoresce with a pale yellow colour. The reproductive organs of all spiders which have not yet functioned show no fluorescence. The light yellow pigment in the male and the ancestral female butterfly, *Papilio dardanus* fluoresces, whilst the mimetic forms of female do not fluoresce.[182]

Blood and Sera.

Much work has been carried out on the examination of blood and the elements contained in it, and monographs have appeared by M. Borst and H. Königsdörffer [41] and by C. Dhéré [99] in which is collected the work of various investigators on porphyrins.

Sera from Human Blood.—O. Reche [121, 122] has examined sera

from human blood in transmitted and incident, filtered and unfiltered ultra-violet light, using layers 7 mm. and 1 mm. thick. The fluorescence spectra of sera from normal healthy persons were not particularly intense, and were various shades of grey-blue and grey-green in colour, according to the blood group. However, he examined 106 cases in his clinic, and found a great variation in the colour and strength of the fluorescence according to the nature of the malady (*e.g.*, yellow, blue, violet and red, etc.). There appears to be no correspondence between the fluorescence shown by the two layers of different thickness, and that of one

Malady.	7 mm. Layer.	1 mm. Layer.	Intensity.
Syphilis	Yellow	Yellow-green	Bright
Gastritis	Yellow	Yellow	Bright
Angina	Blue-grey	Green	Dull
Influenza	Yellow-white	Brown-grey	Dull
Aortitis	Light blue	Brown-grey	Bright
Rheumatism	Blue-grey	Violet	Bright
Nephritis	Yellow	Yellow	Dull
Arterio sclerosis	Light-blue	Blue-green	Bright

cannot be forecasted from that of the other; in diagnosis, therefore, both must be taken into account. The above table are selected examples.

The spectral data are also given, *e.g.*, 3020 Å., and other lines which are normally dark often become quite bright, while others become less bright, although this is less usual. Reche has applied the term " Fluorescence Serology " to this work, and he states that the colours are not due to the presence of foreign material or bacteria, or to the presence of drugs circulating in the blood, but to changes in the sera themselves. Fluorescence diagnosis should therefore have great advantages, especially for advance diagnosis of diseases such as carcinoma (*vide supra*). An instance is provided by the work of A. Achard and A. and J. Bouchard [157] who found that the fluorescence of uranine is unaffected by the addition of sera from perfectly healthy horses or men, but that sera from cancer subjects reduced it in intensity.

Another worker in this direction is W. Buchloh [123] who has confirmed the above findings in 126 patients, and has endeavoured to put this branch of the work on a quantitative basis by measuring

the fluorescence with a Pulfrich step-photometer and employing the Ostwald colour chart and cones (see p. 66) to express numerically the colours and intensities. He found that the normal sera obtained after removing red corpuscles by centrifuging fluoresced in rays of wave-length between 4650 and 4930 Å.; 4750 Å. was the usual wave-length, but the presence of fat raised it (*cf.* Pudor [162]).

Leucocytes in the blood may be examined by the fluorescence method, and two varieties are then visible, namely, a granular leucocyte with a fairly strong fluorescence, and a transparent leucocyte with a weaker fluorescence; the nuclei of these leucocytes appear dark under the lamp. F. Plaut and K. Bossert [156] obtained a pigment substance from the sera of human and rabbit bloods, the green fluorescence of which resembled that of a flavin and which was soluble in chloroform in the presence of alkali, and was stable to acids or bromine. T. Desmonts [186] was able to show that less than 0·1 per cent. of the red cells of normal human blood are fluorescent. This number increases during the week after a hæmorrhage, but returns to the normal value again in 3 weeks. The number of fluorescent cells is related to the porphyrin content of the blood (see below).

Porphyrins.—The fluorescence of *hæmatoporphyrins* was first noted by Hoppe-Seyler [42] in 1880, while in 1911 the fact that hæmatoporphyrin is more fluorescent in alkaline solution than in acid solution was noted by C. Dhéré and Sobolewski,[43] and has since been applied in medico-legal work [76] (see p. 231). It appears that the presence of iron affects the fluorescence of hæmoglobin and its derivatives, for the red blood-substance examined directly under the lamp does not fluoresce, although when it is freed from iron, a red fluorescence is obtained; and it may be noted here that this group of substances and the chlorophylls are the only two known classes of compounds which give such a strong and characteristic red colour.[78]

The change of the fluorescence colour with the pH value of the solution containing natural or artificial porphyrins, as shown by Dhéré and his co-workers,[44–48, 99] is quite general (see pp. 248, 264, 310). They have examined proto-, uro- and coproporphyrins and also the artificial meso-, hæmato-, etio- and isoetioporphyrins in pyridine solution. With the proto-, meso-, and etio-compounds some difficulty was encountered, owing to the fact that they are

only slightly soluble in alkaline solution. In the original papers photographs of the fluorescence spectra, the limiting concentrations (which may be so small as 1 : 10^8), and the wave-lengths of the emitted light are given ; the metallic derivatives of the hæmatoporphyrin and their fluorescence spectra were also examined photographically. H. Fink and K. Weber [49] determined the pH value at which the intensity is a minimum, and obtained values of 3·9, 3·3, and 4·4 for copro-, uro-, and hæmatoporphyrin, respectively ; in many cases these are near the isoelectric point.

In further papers by H. Fink and W. Hoerburger [124–127] the influence of pH value on the fluorescence of porphyrins is discussed. They examined the pH-fluorescence curves of proto-, meso-, deutero-, hæmato- porphyrins and of the tetrachloro- and tetrabromo-derivatives of meso-porphyrin, making a total of 34 porphyrins. They find that the fluorescence, like the optical activity, colour and light-absorption is a function of the constitution, and that the main factor determining the form of the curve is the number of acid groups and their positions.[191] Other substituents, except halogens, appear to have very little influence.

The curves also assist the differentiation of the various porphyrins on the basis of the number of carboxyl groups present, and this applies particularly to etioporphyrin and its mono-, di-, tetra- and octa-carboxylic acids. The pH value at which the fluorescence is a minimum corresponds with the isoelectric point, and this is displaced to the acid side with an increase in the number of carboxyl groups present. Irregularities in the curve are most conspicuous on the alkaline side of the curves, except with etioporphyrin which contains no carboxyl groups ; the influence appears to be due chiefly to the dissolved pigment. In later work [125] the pH-fluorescence curves for the 4 isomeric coproporphyrins and the 3 uroporphyrins are given, and the influence of temperature, concentration, solubility and the presence of inorganic salts is discussed.

These workers have also isolated a porphyrin from normal human urine,[128] and by means of the pH-fluorescence curve have identified it as the copro-1-porphyrin ; in a further example [124] they identified a crystalline material as a uroporphyrin. P. Medinger [129] also recommends the fluorescence method as a means of identifying the porphyrins, and J. T. Brugsch and

A. Keys [192] have obtained successful results by extracting the porphyrin in a mixture of acetic acid and ether, and matching the red fluorescence produced by a solution of the extract in 5 per cent. hydrochloric acid.

M. Wagenaar [130] (see Legal Work, p. 248) has used the fluorescence of hæmatoporphyrin for the detection of blood; hæmatin is non-fluorescent, but if the iron present is removed by treatment with concentrated sulphuric acid, then a fluorescence is visible and the colour changes to violet with alkalis. H. Bierry and B. Gouzon [131] have obtained evidence of the existence of blood protoporphyrin from the fluorescence spectrum of the stannous complex prepared by the action of stannous chloride on hæmatin or hæmoglobin. Another test for hæmin is the reduction of the intensity of the fluorescence produced in the presence of hydrogen peroxide, on addition of benzoquinone.[187]

H. Hellström [132] has investigated the relationship between the fluorescence and the structure of the porphyrins. On exposure of a solution of etioporphyrin in pyridine to sunlight for 2 to 3 days, photoporphyrin was obtained, which gave a characteristic spectrum in the red region, with bands at 6517, 5268, 5018, and 4919 Å., corresponding with a change in both the constitution and the fluorescence spectrum. S. Rafalowski [133] ascribes the weakening of the fluorescence of porphyrins on exposure to ultra-violet light to the formation of and action of acids.

Porphyrin, formed by micro-organisms from hæmoglobin, is found in the mouths of some humans, and is produced during the metabolism of certain anærobic bacteria. If blood is seeded with these bacteria a red fluorescence develops around the colonies. E. Derrien and J. Turchini [50-55] found that the red fluorescence produced from the "Harder" glands of rats, abscesses, the amniotic fluid of sheep, the quills of young porcupines and pigeons, and also from the teeth of some young mammals, such as the dog, pig, etc., is due to porphyrin. It is interesting to note here, however, that the "Harder" glands of numerous other vertebrates do not show this fluorescence. Sorby [56] and others have mentioned the reddish fluorescence of eggshells (see p. 163).

C. H. Dhéré and C. Baumeler [57] consider that a porphyrin exists in the skin of the slug *Arion empiricorum*, and H. Stübel

formerly thought that porphyrin existed in the skin of the earthworm, a conclusion disputed by R. Keller.[22] Further histological work, by Derrien,[58] has been the distinction of parasitic worms in hedgehogs and swine.

Policard [59-61] has studied the red fluorescence of certain *tumours* and he ascribes it to the presence of hæmatoporphyrins; he has also used the lamp for the detection of porphyrins in the *urine* after the administration of certain drugs, for example, sulphonal. The red fluorescence of the urine under the lamp showed the presence of porphyrin, and the presence of urobilin was also detected by extraction with chloroform and treatment of the extract with zinc acetate, when a striking green fluorescence was obtained (*vide infra*). The porphyrin-content of urine may be determined with an error of 2 per cent. without the use of a standard comparison solution [197]; a fluorescence photometer has been evolved for this purpose by A. Thiel,[196] and although the intensity of the fluorescence is not proportional to the porphyrin content, the latter may be deduced from the data supplied by Thiel.

R. Fabre [62] by similar investigations found hæmatoporphyrin in the *bile*; in conjunction with Simonnet [63-65] he examined the liquid spectroscopically and found its spectrograph to be identical with that obtained using the "Harder" gland of the rat, the maximum intensity occurring, according to Bayle and his co-workers (see p. 254), at 6350 Å.

When sulphonal is administered with the food, the hæmatoporphyrin is so rapidly eliminated in the urine that it cannot be detected in the blood. Fabre and Simonnet [63-65] and also Hausmann [66] have applied the method to the detection of hæmatoporphyrin in the urine, to other cases of poisoning, and to certain maladies, and H. Langecker [67] has employed it in cases of lead poisoning.

The synthesis of coproporphyrin by yeast cells has been followed by R. M. Mayer [68] who used the lamp to detect the characteristic red fluorescence. E. J. Bigwood and his co-workers [134] have worked on the fluorescence of concentrated solutions of oxidised cytochrome-C from yeast. This had a marked red fluorescence (6405-6500 Å.) due to the cytochrome itself and not to the presence of a derived porphyrin, which disappeared on reduction or dilution. H. Hellström [69, 70] has

examined the absorption and fluorescence spectra of the porphyrin in solutions of ether, hydrochloric acid or potassium hydroxide, and suggests that a relationship exists between the colour and constitution; thus, tetrabromo meso-porphyrin gives a red fluorescence not shown by the chloro compound. R. Fikentscher [135] has examined the absorption and fluorescence spectra of extracts of fossilised crocodile fæces and has identified coproporphyrin in them. He has also investigated in a similar way the porphyrin content of human amniotic liquid.[136]

The fluorescence of *urobilin* has already been mentioned (*vide supra*, and p. 264) and the work has been carried a stage further by C. Dhéré and J. Roche,[71] who studied the fluorescence spectra of a number of these compounds. The substances were examined in the solid state and in solution in ethyl alcohol, and the absorption spectra were also noted. Some of the results are shown in Table 14.

TABLE 14.

Material (Dry).	Fluorescence.	
	< 425 mμ.	365 mμ.
Bilirubin	Dark red	Dark red
Mesobilirubin	Vivid red	Vivid red
Mesobiliviolin	Nil	Nil
Mesobilirubinogen	Vivid red	Red
Urobilin	Very vivid red	Vivid red
Hydrobilirubin	Nil	Nil

Alcoholic solutions of mesobiliviolin and mesobilirubinogen gave a yellowish fluorescence after the addition of zinc acetate solution, but in the case of urobilin, the same treatment developed a bright green fluorescence. In alcoholic solutions of the complexes formed by mesobiliviolin and mesobilirubinogen with mercuric chloride, the lines of the fluorescence spectra are concentrated chiefly in the orange region. With urobilin and hydrobilirubin, however, under the same conditions, there is no apparent fluorescence. Finally, C. Dhéré [99, 137–146] has published many papers on the fluoresence of pigments of biological importance, *e.g.*, the porphyrin in the skin of the *Lumbricus terrestris*,[141]

mycoporphyrins,[142] eggshells [144] (see Foods, p. 163), coproporphyrins,[146] phylloerythrins,[140] bonellins [138] and of the phycocyanins,[145] etc., for details of which the reader is referred to the original papers; the reaction for copper described on page 199 may also be used as a test for urobilin, and fluorescence methods have been used to determine it in excreta.[177, 195]

In another paper A. Policard [72] discusses the results obtained by the examination of *human ovaries* under the fluorescence microscope. The organ, which should be fresh, is cut with a good microtome and fixed in formalin, when the stroma of the ovary appears white with a bluish tinge. In the middle of the cortical layer this tinge is more bluish, but the translucency is less. The artesic follicles bounded by a yellowish line stand out clearly, and the follicular cavity appears to be filled with a bluish vitreous transparent mass. The corpora albincantia are intense blue and contrast strongly with the white of the stroma. In the yellow cystic bodies the luteinic layer has a granular and clear yellow appearance. The region of the follicles (which includes the blood) are black and opaque, but the luteinic bodies stand out with a yellow tinge and the fibrous and hyaline bodies have a characteristic translucent blue appearance.

Drugs in Body Fluids.

Drugs administered to a patient can often be detected in the urine and saliva by means of the lamp, and a number of important papers deal with this operation (see also p. 117). Details are given on page 252 of the results obtained by Bayle and his co-workers in their experiments with hydrastine, quinine sulphate, aspirin, etc. F. Caujolle [73] mentions the detection of quinine in bile after an intravenous injection, and Frank [81] has confirmed Bayle's method for the estimation of quinine in saliva.

Radley has found that aspirin may be detected in the *urine* of a subject to whom it has been administered, by making it alkaline and examining it under the lamp, when a strong violet fluorescence is visible. The strong blue fluorescence of urine of subjects suffering from heavy cold has also been noted by this worker, and according to P. Niederhoff and G. Holland [147] the fluorescence of urine in cases of malaria or high fever is a strong

sky blue. In cases of apiol poisoning M. Herrmann [148] has detected the poisonous triorthocresyl-phosphate by its intense blue fluorescence. K. Kramer [149] quotes the experiment of Tappiner who noted the excretion of eosin for 7 days after subcutaneous and intravenous injections, and the urinary excretion of eosin for 14 days after.

Acridine derivatives in urine are recognisable from the intense green fluorescence produced on adding 2 drops of a 20 per cent. solution of sodium caffeine benzoate in water to 5 c.c. of urine.[172] As G. Discombe [173] has shown, however, if fluorescein is present in the urine, a green fluorescence results without addition of any reagent, and this upsets the test. The fluorescein, which has an absorption band similar to that of urobilin, may be separated by extraction with acidified amyl alcohol, the resulting extract being itself extracted with ethyl alcohol and dilute alkali. The fluorescence of the fluorescein is visible in the final extract, but the eosin test (see p. 195) may be used to confirm its presence. Attempts have been made to determine bile acids in urine from the intensity of the fluorescence produced on addition of sulphuric acid, but the method is complicated by the similar fluorescence produced by other substances which may be present.[174]

A very important paper on this subject is due to R. Joachimovits [74] in which the detection of various drugs passing into the human milk and amniotic liquid is discussed. The chemical investigation of such cases is difficult, and useful information may often be obtained by fluorescence methods. These are especially important from the point of view of tracing the effect of medicine, administered to the mother, on the child. Joachimovits also emphasises the importance of the liquid medium employed, as it may give rise to a fluorescence which masks or alters that of the substance sought. For example, many drinking waters have a distinct fluorescence (see p. 378), although distilled water is non-fluorescent. The fluorescence of ether or alcohol is negligible unless, in the latter case, 1 per cent. or more of acetone is present, when a bluish fluorescence results.

A blue-green fluorescence which resembles that obtained from lactose is given by the fat-free filtrate from *cow's milk*, and this is due to an unknown constituent which is absent from human milk. This substance is not removed by extraction with ether

and may be due to a coumarin derivative. As mentioned on page 160, the fluorescence of milk decreases on ageing, and suggestions that this phenomenon is connected with oxidation are confirmed by the fact that treatment with bromine or with potassium permanganate accelerates the fading process.

Alkaloids (see also pp. 118 and 313).—According to G. Klein [75] the limiting concentrations for which fluorescence is visible under optimum conditions are: quinine, $1 : 1{\cdot}4 \times 10^7$; hydrastinine, $1 : 1{\cdot}26 \times 10^6$, or, if the fluorescence microscope is used, the limiting concentration for the latter becomes $1 : 1{\cdot}2 \times 10^8$. A modified Stass-Otto process is used for the extraction of alkaloids and hydroxymethyl anthraquinone, the proteins being precipitated with a four-fold volume of alcohol, the filtrate evaporated, and the residue extracted with dilute tartaric acid; this is then made just ammoniacal, and the fat and free alkaloids are removed by shaking with an equal volume of ether, the alkaloids being finally separated from the fat by extraction with a 1 per cent. solution of tartaric acid, and this acid extract should be examined under the lamp.

One part of quinine in $1{\cdot}4 \times 10^7$ is detectable in *mothers' milk* even 2-3 hours after administration, and the fluorescence also appears in the amniotic liquid and urine of the infant.[185] The fluorescence is destroyed by 80 per cent. hydrochloric acid, but is modified and intensified by sulphuric acid. In certain cases plasmoquine is substituted for quinine; it is an alkyl amino-methoxy quinoline derivative and fluoresces with a strong yellow-green colour, although the fluorescence does not appear in the milk to any great extent. Quinine in blood may be determined by the method of J. Kaiser.[163] The citrated blood is made alkaline and heated, and then extracted with chloroform. The portion of the extract soluble in acidified ether is extracted with petroleum spirit, and in this way the quinine is obtained free from any fats or colouring matters from the blood. The determination is carried out in filtered ultra-violet light by comparison with a mixture of Pyrrole Blue and Patent Green, the fluorescence of which simulates that of quinine sulphate closely, on an optical bench which facilitates matching by allowing the distance between the light source and the comparison tube to be varied. The range of the method is 0·5 to 8 γ of quinine per c.c., and the mean recorded error is 4 per cent. H. Kaiser and

his co-workers[184] have evolved a method for the detection of acetic acid in urine by fluorescence analysis.

The thalleioquin reaction for quinine may be sensitised with the aid of ultra-violet light, and it is then particularly suitable for the detection of quinine in urine; in this way, the green fluorescence is detectable at concentrations of 1 : 500,000 instead of only 1 : 20,000. M. Haitinger's procedure[190] is to place 1 drop of the test solution on a filter paper, which is held in bromine vapour while moist; the fluorescence brightens and then starts to fade, and at this point the paper is held in ammonia vapour, when the fluorescence changes from blue to greenish-yellow.

Hydrastinine appears in the milk 4 hours after a dose of 15 drops or 1½ hours after intramuscular injection, but disappears after 8 hours. In this case the bluish-white fluorescence is enhanced by the addition of hydrochloric acid. Small quantities of bromides in human milk may be determined by the fluorescein-eosin method (see p. 195).

With "Gynergen" (a solution of ergotamine in tartaric acid) a pale blue opalescent fluorescence is observed, which is enhanced by sulphuric acid and destroyed by hydrochloric acid. Similar behaviour is observed with ergotoxine, the reaction being visible in the milk 2 hours after administration.

Cotarnine appears in the milk after 1-10 hours as a yellow-brown fluorescence and is visible in a dilution of $1 : 6 \times 10^6$. Hydroxymethyl anthraquinone was given in the form of istizin, rhubarb, aloe and senna, both to human beings and to cows, but the resulting fluorescence is not so easily distinguishable as those of the alkaloids mentioned above. The light green fluorescence of aloin may be increased by heating with borax, especially if the milk is first cleared with alcohol as already described, and $1 : 10^7$ parts is visible; rheum, however, gives negative results. Cocaine fluoresces yellow-green in aqueous solution, but novocaine or arbutin have at most only a weak surface-fluorescence, and capillary analysis (p. 59) is recommended in such cases. That the results should be interpreted with caution is evident from 3 cases cited by C. Griebel.[184] One refers to modifications in the fluorescence of human milk due to istizin (see above), and another to an abnormal red fluorescence which was traced to a protein differing from casein or lactalbumin; in the third case a genuine sample of human milk was found to have a yellow

fluorescence. Other abnormal results are attributed to changes in fluorescence which occur in milk on exposure to light; and to milk from a vegetarian mother.

J. Sutro and M. S. Burman [150] have used the fluorescence of eosin to render the biliary system visible. The eosin was injected into the blood-stream of rabbits either intravenously or intracardiacally and the gall bladder in the opened abdomen was irradiated. The whole biliary system was thus seen with a deep golden-yellow fluorescence including the gall bladder, its tributary hepatic and cystic ducts, and the common duct entering the duodenum at the ampulla; the large intestines were less visible. The surface of the kidneys had a speckled gold appearance, and after several hours the ureters and finally the urinary bladder became faintly fluorescent. A dye which has a strong fluorescence and is quickly eliminated by the kidneys would be a valuable adjunct to the surgery of the ureters. H. Havlicek [151] has used the lamp similarly for abdominal surgery, and by staining with trypaflavin or rivanol he was also able to detect marks from old operations.

Some work of medical interest on ergosterol (see also Foods, p. 148) is due to Nagelschmidt,[152] who found that after irradiation its strong blue fluorescence is lost, but that it then showed antirachitic properties which the fluorescent form did not possess. An ultra-violet test for A-avitaminosis is reported by Mouriquand and his co-workers.[160] K. G. Stern [153] has noted that animal and vegetable catalase preparations have an intense green fluorescence, and that chemical or physical treatment, which irreversibly decreases the catalase activity, corresponds with a change in colour towards the blue portion of the spectrum and with a decrease in the intensity of the fluorescence, which finally is extinguished.

REFERENCES.

[1] Brewster, *Phil. Mag.*, 1867.

[2] Helmholtz, *Physiol. Optik.*, 1896, 284, 306.

[3] Köhler, *Zeit. wiss. Mikros.*, 1904, **21**, 163.

[4] Hess, *Archiv. vergl. Ophthalmol.*, 1911, **2**, 3.

[5] Hess, *Pflüger's Arch. Physiol.*, 1911, **137**, 339.

[6] Goldstein, *Phys. Zeit.*, 1911, **12**, 614.

[7] Kowalski, *Bull. Soc. Fribourgeoise d. Sciences Naturelles*, 1911, **19**, 32.

[8] H. Stübel, *Pfluger's Arch. Physiol.*, 1911, **142**, 1.

[9] J. Turchini, *Compt. rend. Soc. Biol.*, 1925, **93**, 1088; *Ber. ges. Physiol.*, 1927, **38**, 366, 657.

[10] J. Turchini and H. Havant, *Ber. ges. Physiol.*, 1926, **94**, 1535.
[11] O. Duboscq and J. Turchini, *ibid.*, 1925, **93**, 1090.
[12] E. Merker, *Zool. Jahrb. Abt. Allgem. Zool. u. Physiol.*, 1928, **45**, 535; 1929, **46**, 297, 483.
[13] J. C. Moltram and E. A. Cockayne, *Chem. News*, 1920, **132-133**, 247.
[14] W. S. Andrews, *Gen. Elect. Rev.*, 1925, **28**, 659.
[15] P. Wels, *Pflüger's Arch. Physiol.*, 1929, **223**, 369.
[16] E. Newton Harvey and P. A. Snell, *Jour. Gen. Physiol.*, 1931, **14**, 529.
[17] E. N. Harvey, *American Jour. Physiol.*, 1926, **77**, 555.
[18] E. N. Harvey, *ibid.*, 1925, **72**, 218.
[19] P. R. Peacock, *Lancet*, 1925, **209**, 369.
[20] J. Margarot and P. Devèze, *Bull. Soc. d. Sci. méd. et biol. de Montpellier et du Languedoc Méditerranéen*, 1925, **6**, 375; *Zentralbl. Hautkrankh.*, 1926, **19**, 385; 1930, **32**, 205; *Ann. Dermat.*, 1929, **10**, 581.
[21] H. Goodman, *Dermatol. Wochschr.*, 1928, **87**, 957.
[22] P. Keller, *Dermatol. Zeit.*, 1926, **49**, 33.
[23] H. Fuhs, *ibid.*, 1927, **50**, 16.
[24] H. Lewin, *Dermatol. Wochschr.*, 1926, **82**, 850.
[25] J. Meyer and Saidmann, *ibid.*, 1926, **82**, 404.
[26] Rorburgh, *Dermatol. Zeit.*, 1928, **51**, 372.
[27] Nogier, *Zentr. Hautkrankheit*, 1926, **19**, 386; *Arch. élec. Méd.*, 1925, **53**, 97.
[28] N. Gray Hill, *Brit. Jour. Actinotherapy*, 1928, **3**, no. 5; *Brit. Jour. Children's Diseases*, 1928, **25**, 54.
[29] A. A. Hymans van den Bergh and Hyman, *Konigkl. Akad. Wetensch. Amsterdam wisk. natk. Afd.*, 1927, **36**, 1096.
[30] S. Bommer, *Klin. Wochschr.*, 1927, **6**, 1142, 1705; *Acta Derm. Venereol.*, 1929, **10**, 253, 391.
[31] S. Bommer, *Dermatol. Wochschr.*, 1926, **82**, 637.
[32] E. L. Kennaway and I. Hieger, *Brit. Med. Jour.*, 1930 (i), 1044.
[33] L. Lombardi, *Boll. della R. Staz. sper. di Gelsicolt e Bachilolt. Ascoli Miceno*, 1928, **7**, 35.
[34] A. Tonon, *Boll. di sericultura*, 1927, **34**, 51.
[35] Sergio Beer, *Boll. Soc. Ital. Biol. Sperim.*, 1928, **6**, 162.
[36] A. Policard and A. Paillot, *Compt. rend.*, 1925, **181**, 378.
[37] M. T. Ertrogroul, *ibid.*, 1928, **1**, 865.
[38] A. Segitz, *Der Spinner und Weber*, 1926, no. 59.
[39] J. Kodahira, *Jour. Sericulture, Japan*, 1927, **1**, 10.
[40] J. Turchini and J. Millot, *Ber. ges. Physiol.*, 1926, **36**, 368; *Compt. rend. Soc. Biol.*, 1926, **94**, 171.
[41] M. Borst and H. Königsdörffer (Publishers, S. Hirzel, Leipzig), 1929.
[42] Hoppe-Seyler, *Zeit. physiol. Chem.*, 1880, **4**, 201.
[43] C. Dhéré and S. Sobolewski, *Compt. rend. Soc. Biol.*, 1911, **63**, 511.
[44] C. Dhéré and E. Bois, *Compt. rend.*, 1926, **183**, 321.
[45] C. Dhéré, A. Schneider and T. van d. Bom, *ibid.*, 1924, **179**, 351.
[46] C. Dhéré, A. Schneider and T. van d. Bom, *ibid.*, 1924, **179**, 1356.
[47] T. van d. Bom, "Untersuchung uber die Fluorescenz des Hämatoporphyrins," Dissertation, 1924, Freiburg.
[48] C. Dhéré and J. Aharoni, *Compt. rend.*, 1930, **190**, 1499.
[49] H. Fink and K. Weber, *Naturwissenschaft*, 1930, **18**, 16.
[50] E. Derrien and J. Turchini, *Compt. rend. Soc. Biol.*, 1924, **91**, 637.
[51] E. Derrien and J. Turchini, *ibid.*, 1925, **92**, 1028.
[52] E. Derrien and J. Turchini, *ibid.*, 1925, **92**, 1030.

[53] E. Derrien and J. Turchini, *Compt. rend. Assoc. Anatomistes*, 1925, **20**, 177.

[54] E. Derrien, *Bull. Soc. Chim. Biol.*, 1916, **8**, 218.

[55] E. Derrien, *Compt. rend. Soc. Biol.*, 1924, **91**, 634; *Bull. Soc. Chim. France*, 1924, **35**, 391, 687.

[56] Sorby, "Lehrbuch der Physiologisch Chemie," 9th ed., p. 502.

[57] C. Dhéré and C. Baumeler, *Compt. rend. Soc. Biol.*, 1928, **99**, 726.

[58] E. Derrien, *Compt. rend.*, 1927, **184**, 480; with C. Bénoit, *Arch. Soc. Sci. Med. Biol., Montpellier*, 1929, **8**, 456; 1929, **9**, 510; *Bull. Soc. Chim. France*, 1929, **45**, 689.

[59] A. Policard, *Compt. rend. Soc. Biol.*, 1924, **91**, 1423.

[60] A. Policard and A. Leulier, *ibid.*, 1924, **91**, 1422.

[61] A. Policard, *Bull. d'Histol.*, 1925, **2**, no. 5.

[62] R. Fabre, *Bull. Soc. Chim. Biol.*, 1925, **7**, 1024.

[63] R. Fabre and H. Simonnet, *Compt. rend. Soc. Biol.*, 1925, **92**, 1026.

[64] R. Fabre and H. Simonnet, *Jour. Pharm. et Chim.*, 1925, **2**, 225.

[65] R. Fabre and H. Simonnet, *Bull. Soc. Chim. Biol.*, 1925, **7**, 1129.

[66] W. Hausmann, *Strahlentherapie*, 1913, **3**, 112.

[67] H. Langecker, *Zeit. physiol. Chem.*, 1921, **150**, 1.

[68] R. M. Mayer, *ibid.*, 1928, **177**, 47.

[69] H. Hellström, *Zeit. Phys. Chem.*, 1931, **12**, 353.

[70] H. Hellström, *ibid.*, 1931, **14**, 17.

[71] Ch. Dhéré and J. Roche, *Compt. rend.*, 1931, **193**, 673; *Bull. Soc. Chim. Biol.*, 1931, **13**, 987 (Urobilin group); C. Dhéré, *Compt. rend. Soc. Biol.*, 1930, **103**, 371 (Bilirubin derivatives); *Arch. Int. Pharmacodynamic Therap.*, 1930, **38**, 134 (Bilirubin derivatives); C. Dhéré and M. Fontaine, *Compt. rend.*, 1931, **192**, 1131; *Ann. Inst. Oceanogr.*, 1931, **10**, 249 (Algoid pigments); *Compt. rend. Soc. Biol.* 1931, **105**, 843 (Bonellins); E. Bachrach and C. Dhéré, *ibid.*, 1931, **108**, 385 (Diatoms and the chlorophyll pigments).

[72] A. Policard, *Compt. rend.*, 1924, **179**, 1287.

[73] F. Caujolle, *Bull. Soc. Chim. Biol.*, 1930, **12**, 299.

[74] R. Joachimovits, *Monats. für Geburtshilfe u. Gynäkologie*, 1929, **83**, 42.

[75] G. Klein, "Histochemie," 1928.

[76] A. Treibs, *Zeit. physiol. Chem.*, 1932, **212**, 33.

[77] E. Furreg and F. Querner, *Acad. Sci., Vienna*, July, 1932; *Akad. wiss. Wien, math.-naturw. klasse, Akad. Anz.*, No. 11, 1929; *Zeit. Wiss. Zool.*, 1931, **136**.

[78] S. Rafalowski, see *Brit. Chem. Abst. A.*, 1933, 209.

[79] S. Hoshijima, *Inst. Phys. Chem. Res. Tokyo*, 1933, **20**, 109.

[80] A. Hadjioloff and T. Kresteff, *Compt. rend. Soc. Biol.*, 1929, **102**, 779; 1931, **106**, 662, 663; *Bull. Histol. Applic.*, 1929, **6**, 37; 1932, **9**, 153.

[81] Frank, *Chem. Tech. Rundschau*, 1930, **45**, 841.

[82] E. Truc, *Compt. rend. Soc. Biol.*, 1928, **99**, 1847.

[83] S. Bommer, *Dermatol. Wochschr.*, 1934, **98**, 413.

[84] A. Furniss, *Med. World*, 1933, **38**, 513.

[85] S. Hoshijima, *Sci. Papers Inst. Phys. Chem. Res. Tokyo*, 1933, **20**, 109; 1933, **21**, 15.

[86] H. Goodman, *N. York State Jour. Medicine*, 1929, **29**, 542.

[87] H. Goodman, *Phys. Therapeutics*, 1928, **46**, 79; *Clin. Med. Surg.*, 1929, **36**, 44.

[88] H. Goodman, *Jour. Phys. Ther.*, 1928, **4**, 468; *ibid.*, 1928, **5**, 203.

[89] H. Goodman, *Brit. Jour. Derm.*, 1928, **40**, 105.

[90] P. Ellinger and W. Koschara, *Nature*, 1934, **133**, 553; *Ber.*, 1933, **66**, 315, 808; *Zeit. physiol. Chem.*, 1934, **229**, 103.

[91] J. B. Bateman, *Nature*, 1934, **133**, 860.
[92] M. Haitinger and H. Hamperl, *Zeit. Mikros. Anatom. Forsch.*, 1933, **33**, 193.
[93] P. Ellinger and A. Hirt, *Zeit. Anat. Entwicklungsgesch.*, 1929, **90**, 791; *Umschau*, 1930, **34**, 136; Abderhalden's "Handbuch der Biologischen Arbeitsmethoden," 1930, **5**, II, p. 1753 (Berlin: Urban and Schwarzenberg).
[94] P. Ellinger and A. Hirt, *Arch. Exp. Path. Pharm.*, 1929, **145**, 193.
[95] P. Ellinger and A. Hirt, *ibid.*, 1930, **150**, 285.
[96] W. Hartoch, *Zeit. ges. expt. Med.*, 1931, **79**, 538; *Klin. Wochschr.*, 1932, **11**, 1464; 1933, **12**, 942.
[97] A. Grabner, *Umschau*, 1932, **36**, 371.
[98] E. Baum, *ibid.*, 1932, **36**, 418.
[99] Abderhalden's "Handbuch der Biologischen Arbeitsmethoden," 1930, **5**, II, p. 1253; 1933, III, 4, 5 (Berlin: Urban and Schwarzenberg).
[100] A. Roche, *Bull. Soc. Chim. Biol.*, 1932, **14**, 1026.
[101] A. M. Davidson and P. H. Gregory, *Canad. Jour. Res.*, 1932, **7**, 378.
[102] J. Kinnear, *Brit. Med. Jour.*, 1931, No. 3670, 791.
[103] H. McCormich Mitchell, *Med. World*, 1930, **32**, 122.
[104] A. G. Millot Severn, *Med. Officer*, 1929, p. 113.
[105] A. C. Roxburgh, *Proc. Roy. Soc. Med., Derm. Sect.*, 1927, **17**, 80.
[106] P. Vigne, *Marseille Médical*, 1926, **63**, 1765; *Presse méd.*, 1927, **35**, 339.
[107] W. G. Pugh, *Rep. Met. Asylums Board*, 1926, 416.
[108] W. K. Sibley, *Proc. Roy. Soc. Med., Derm. Sec.*, 1927, **22**, 81.
[109] J. W. Cook, *Jour. Chem. Soc.*, 1933, 395.
[110] J. W. Cook, *Nature*, 1932, **130**, 926.
[111] J. W. Cook, I. Hieger, E. L. Kennaway and W. V. Mayneord, *Proc. Roy. Soc.*, 1932, **111B**, 455.
[112] J. W. Cook, *ibid.*, 1932, **111B**, 485.
[113] I. Hieger, *Biochem. Jour.*, 1930, **24**, 505.
[114] B. Grynkraut, *Neoplasme*, 1933, **12**, 5.
[115] E. L. Kennaway, *Jour. Path. Bact.*, 1924, **27**, 234.
[116] E. L. Kennaway, *Brit. Med. Jour.*, 1925, **2**, 1.
[117] E. L. Kennaway, *Biochem. Jour.*, 1930, **24**, 497.
[118] S. Beer, *Ber. ges. Physiol.*, 1929, **48**, 484.
[119] S. Beer, *Boll. Soc. Ital. Sper.*, 1930, **5**, 1113.
[120] S. Beer, *Ber. ges. Physiol.*, 1932, **63**, 695.
[121] O. Reche, *Zeit. f. Rassenphysiol.*, 1931, **4**, 97; *Arch. Phys. Therapy*, 1934, Oct.
[122] O. Reche, *Münch. med. Wochschr.*, 1931, **78**, 1591, 2051.
[123] W. Buchloh, *Zeit. f. Rassenphysiol.*, 1933, **6**, 166.
[124] H. Fink and W. Hoerburger, *Zeit. physiol. Chem.*, 1931, **202**, 8.
[125] H. Fink and W. Hoerburger, *ibid.*, 1933, **218**, 181.
[126] H. Fink and W. Hoerburger, *ibid.*, 1933, **220**, 123.
[127] H. Fink and W. Hoerburger, *ibid.*, 1934, **225**, 49; 1935, **232**, 28; **236**, 136.
[128] H. Fink and W. Hoerburger, *Naturwiss.*, 1934, **22**, 292.
[129] P. Medinger, *Ann. Chim. Analyt.*, 1933, **15**, 241.
[130] M. Wagenaar, *Pharm. Weekblad*, 1934, **71**, 478.
[131] H. Bierry and B. Gouzon, *Compt. rend.*, 1934, **198**, 615.
[132] H. Hellström, *Arkiv. Kemi. Min. Geol.*, 1933, **11B**, 5; through *Brit. Chem. Abst.* A., 1934, 724.
[133] S. Rafalowski, *Zeit. Phys.*, 1931, **71**, 798.
[134] E. J. Bigwood, J. Ansay and J. Thomas, *Compt. rend. Soc. Biol.*, 1933, **112**, 1584.

[135] R. Fikentscher, *Zoöl. Anzeig.*, 1933, **103**, 289.
[136] R. Fikentscher, *Arch. f. Gynäkologie*, 1933, **154**, 129.
[137] C. Dhéré, Abderhalden's "Handbuch der Biologischen Arbeitsmethoden," II, 3, 4, 1933 (Berlin : Urban and Schwarzenberg).
[138] C. Dhéré, *Compt. rend. Soc. Biol.*, 1932, **110**, 1254.
[139] C. Dhéré, *Ann. Physiol. Physicochem. Biol.*, 1932, **8**, 760.
[140] C. Dhéré, *Compt. rend.*, 1932, **195**, 336.
[141] C. Dhéré, *ibid.*, 1932, **195**, 1436.
[142] C. Dhéré, *ibid.*, 1933, **197**, 948.
[143] C. Dhéré, *Compt. rend. Soc. Biol.*, 1933, **112**, 1129.
[144] C. Dhéré, *ibid.*, 1933, **112**, 1595.
[145] C. Dhéré, *Compt. rend.*, 1934, **198**, 126.
[146] C. Dhéré and A. Roche, *Compt. rend. Soc. Biol.*, 1933, **114**, 449.
[147] P. Niederhoff and G. Holland, *Klin. Wochschr.*, 1933, **12**, 1184.
[148] M. Herrmann, *Samml. von Vergiftungsf.*, 1933, **4**, 360.
[149] K. Kramer, *Virchow's Arch. f. Path. Anat.*, 1929, **274**, 215.
[150] J. Sutro and M. S. Burman, *Jour. Amer. Med. Assoc.*, 1932, **99**, 2024.
[151] H. Havlicek, *Beitr. z. klin. Chir.*, 1931, **154**, 254.
[152] Nagelschmidt, *Amer. Jour. Phys. Therapy*, 1928, **5**, 222.
[153] K. G. Stern, *Zeit. physiol. Chem.*, 1932, **212**, 207.
[154] Kämmerer, *Münch. med. Wochschr.*, 1931, **78**, 1711.
[155] F. Querner and K. Sturm, *Zeit. Mik. Anat. Forsch.*, 1933, **32**, 444 ; *Anat. Anz. Histol. Inst.*, *Wien*, 1934, **78**, 289 ; *Naturwiss.*, 1932, **20**, 908.
[156] F. Plaut and K. Bossert, *Klin. Wochschr.*, 1934, **13**, 450.
[157] A. Achard and A. and J. Bouchard, *Compt. rend.*, 1934, **199**, 908.
[158] H. von Euler and E. Adler, *Zeit. physiol. Chem.*, 1934, **228**, 1.
[159] S. G. Newman, *An. Rept. of Chief M.O. to Board of Education*, 1933, p. 8.
[160] A. Mouriquand, J. Rollet and M. Courbières, *Compt. rend.*, 1935, **200.**, 787.
[161] E. Tiede and H. Chromse, *Ber.*, 1934, **67B**, 1988.
[162] H. Pudor, *Fortschr. d. Medezin*, 1933, **51**, 695.
[163] F. J. Kaiser, *Rec. Trav. Chim. Pays-Bas*, 1938, **57**, 117.
[164] W. Donovan, *Analyst*, 1937, **62**, 609.
[165] P. E. Martin and E. Receveur, *Compt. rend. Soc. Biol.*, 1937, **125**, 663.
[166] G. M. Blech, *Arch. Phys. Therapy*, 1934, Oct.
[167] J. W. Cook, *Chem. Weekblad*, 1935, **32**, 563 ; *Chem. and Ind.*, 1935, **54**, 1022.
[168] C. Sannié, *Biochem. J.*, 1936, **30**, 704.
[169] W. V. Mayneord, *ibid.*, 1936, **30**, 707.
[170] J. B. Bateman, *Biol. Rev. Camb. Phil. Soc.*, 1935, **10**, 42.
[171] H. Barth, *Biochem. Z.*, 1936, **285**, 311 ; O. Rahn, "Invisible Radiations of Organisms" (Berlin : Borntraeger), 1936 ; A. Hollaender and W. D. Claus, *Nat. Res. Counc. Washington*, *Bull.* No. 100, 1937.
[172] G. Ferrari, *Diag. Tec. Lab. Napoli*, 1934, **5**, 928 ; through *Brit. Chem. Abst. A.*, 1936, 362.
[173] G. Discombe, *Lancet*, 1937, **232**, 86.
[174] M. Jenke and F. Bandow, *Zeit. physiol. Chem.*, 1937, **249**, 16.
[175] A. M. Davidson, S. A. Boyd and C. P. Haltalin, *J. Amer. Med. Assoc.*, 1935, **105**, 1262.
[176] A. Furniss, *Medical Officer*, 18/9/37.
[177] H. Opitz and Brehme, *Zeit. Ges. Exp. Med.*, 1924, **41**, 681.
[178] M. Van Lint, *Bull. Soc. Belge Ophthalmol.*, 1935, No. 1 ; *Quartz Lamp*, 1938, **7**, (1-2), 16.

[179] A. Gourévitch, *Compt. rend. Soc. Biol.*, 1938, **127**, 214, 1061.

[180] W. H. Byler, *Jour. Amer. Chem. Soc.*, 1938, **60**, 1247.

[181] M. Oku, *Jour. Agric. Chem. Soc. Japan*, 1934, **10**, 1239, 1253; Y. Kishi and Y. Yokata, *Lignan Sci. Jour.*, 1932, **10**, 500; G. Barbera, *Ann. Chim. Applic.*, 1933, **23**, 501.

[182] G. D. H. Carpenter, *Nature*, 1936, **138**, 243.

[183] G. Brooks, *ibid.*, 1936, **138**, 179.

[184] C. Griebel, *Z. Unters. Lebensm.*, 1936, **72**, 46.

[185] T. A. G. Haanappel, *Pharm. Weekblad*, 1937, **74**, 871.

[186] T. Desmonts, *Sang*, 1938, **12**, 180.

[187] B. Tamamushi and A. Akiyama, *Z. Physikal. Chem.*, 1938, **38B**, 400.

[188] H. Minibeck, *Biochem. Z.*, 1938, **297**, 29, 40, 214.

[189] M. Fontaine and R. G. Busnel, *Compt. rend.*, 1938, **206**, 1679.

[190] M. Haitinger, *Mikrochimica Acta*, 1937, **1**, 1.

[191] F. Bandow and E. J. Klaus, *Zeit. physiol. Chem.*, 1936, **238**, 1.

[192] J. T. Brugsch, *Münch. Med. Woch.*, 1934, No. 40; 1935, **82**, 1803; (with A. Keys), *Proc. Soc. Exp. Biol. Med.*, 1938, **38**, 557.

[193] A. Boutaric and J. Bouchard, *Compt. rend. Soc. Biol.*, 1935, **120**, 293.

[194] H. Kaiser, E. Wetzel and L. Föll, *Sudd. Apoth.-Ztg.*, 1929, No. 100.

[195] C. J. Sutro and M. S. Burman, *Jour. Amer. Med. Assoc.*, 1932, Dec.; *Arch. Path.*, 1933, Sept.

[196] A. Thiel, *Biochem. Z.*, 1938, **298**, 436.

[197] F. Bandow, *Strahlenther.*, 1938, **61**, 664.

[198] H. Druckrey, *Arch. Exp. Path. Pharm.*, 1938, **190**, 184.

ADDITIONAL REFERENCES.

F. P. Fischer, *Koll. Beih.*, 1928, **28**, 333 (Eyes).

C. Sorgenfrei, *Zeit. wiss. Photogr.*, 1929, **27**, 169 (Photography of living tissues).

R. Kuhn, P. György and T. Wagner-Jauregg, *Ber.*, 1933, **66**, 317 (Natural colouring matters).

A. A. Kirstner and M. M. Pantschenkow, *Arch. Schiffs- u. Tropenhygeine*, 1931, **35**, 286 (The course of quinine in the organism).

N. von Jancsó, *Klin. Wochschr.*, 1932, **16**, 689 (Fluorescence microscopy; trypanosomes rendered visible by acridine).

C. A. Rothenheim, *Zentralblat. f. Pharm.*, 1933, **29**, Nos. 1 and 2 (Body fluids, hormones and glands).

P. Wels and M. Jokisch, *Pflüger's Arch.*, 1928, **219**, 738; 1930, **223**, 367, 395; 1931, **228**, 672 (Tissues, cells and sera).

H. H. Tohoku, *Jour. exp. Med.*, 1929, **13**, 524; *Ber. ges. Physiol.*, 1930, **55**, 664; 1932, **69**, 161 (Eye fluids and organs).

Huji Hoseya, *Ber. ges. Physiol.*, 1932, **69**, 161.

A. Policard and M. Pehu, *Bull. d. Histol. Appl.*, 1928, **5**, 266; *Ber. ges. Physiol.*, 1928, **47**, 194 (Detection of rachitic bone).

Y. Yoneyama, *Zeit. ges. exp. Med.*, 1931, **76**, 680 (Urine).

P. Niederhoff and G. Holland, *Klin. Wochschr.*, 1933, **12**, 1184 (Urine).

I. P. Becker and P. Szendrö, *Pflüger's Arch.*, 1931, **228**, 755.

W. H. Wadsworth and E. A. Miserheimer, *Jour. Amer. Med. Assoc.*, 1928, **90**, No. 18.

W. T. Anderson, *Arch. phys. Ther.*, 1930, **11**, 482 (General).

De Grave, *Scalpel*, 1927, **80**, 62 (Dermatology).

Ferrari, *Giorn. ital. Dermat.*, 1927, **68**, 1464 (Dermatology).

M. Jijkerk, *Zentr. Hautkrkh.*, 1928, **27**, 740 (Medical investigation).

D. E. H. Cleveland, *Arch. Dermat.*, 1928, **18**, 368 (Detection of trichophyten).

I. Rothman, *Therapia (Budapest)*, 1929, **6**, 339 (Skin diseases).

H. H. Ray, *Amer. Jour. Child. Dis.*, 1929, **38**, 339 (Aid to diagnosis).

G. H. Klövekorn and O. Gaertner, *Strahlentherapie*, 1931, **41**, 1 (Detection of infected hair).

E. Derrien and C. Bénoit, *Arch. Soc. Sci. méd. e. biol. Montpellier*, 1929, **9**, 510 (Porphyrins, salivary calculi) ; *Bull. Soc. Chim. France*, 1929, **45**, 689, 391 (Copper-containing and other porphyrins).

S. Loos, *Zeit. Stomat.*, 1931, **29**, 1 (Porphyrin formation on the teeth).

J. Körbler, *Strahlentherapie*, 1932, **43**, 317 ; 1931, **41**, 510 (Tumours).

E. Singer, *Science*, 1932, 289 (Fluorescence microscopy of living tissues).

H. Hauser, *Strahlentherapie*, 1929, **33**, 582.

A. A. Hymans van der Bergh, W. Grotepass and F. P. Revers, *Klin. Wochschr.*, 1932, **11**, 1534 (Blood and gall).

R. Fikentscher, H. Fink and E. Emminger, *Virchow's Arch. Path. Anat.*, 1933, **287**, 764 ; *ibid.*, 1931, **279**, 731 (Porphyrins).

H. Pflüger, *Klin. Wochschr.*, 1931, **10**, 572 (Porphyrins).

M. Royer, *Semana med.*, 1932, **39**, 1348 (Estimation of the urobilins).

B. Spetzer and S. Loos, *Jahrb. f. Psychiatre u. Neurologie*, 1934, **50**, 309 (Fluorescence microscope for observing fluids injected in the nerve system).

M. Fontaine, *Compt. rend. Soc. Biol.*, 1934, (i), 1266 (Blood pigments).

P. Ellinger, C. E. Edgar, and N. A. Lucas, Biochem. Soc., 15/3/35 (Porphyrins in urine of rats deprived of vitamin B_2 have a pink fluorescence).

R. Kuhn and T. Wagner-Jauregg, *Ber.*, 1934, **67B**, 1770 (Lactoflavin from liver).

P. Karrer, H. Salomon, K. Schöpp, E. Schlittler, and H. Fritzsche, *Helv. Chim. Acta*, 1934, **17**, 1010 (Lactoflavin).

E. Emminger and G. Battistini, *Virchow's Arch. Path. Anat. Physiol.*, 1933, **290**, 492 ; *Klin. Woch.*, 1933, **12**, 184 (Fluorescence microscopy in cases of poisoning).

K. Franke and A. Scylla, *Zeit. ges. Exper. Med.*, 1933, **89**, 41 (Microscopical investigation of living organisms in ultra-violet light).

R. Inouye, H. Yamazaki, T. Miwa, K. Sakamoto and S. Yamykoski, *Bull. Sericult. Silk Ind. Japan*, 1930, **3**, 3 (Fluorescence of cocoons).

K. Franke and R. Fikentscher, *Klin. Woch.*, 1934, **13**, 285 ; *Münch. Med. Woch.*, 1935, **82**, 171 (Determination of porphyrins by means of fluorescence as an indication of liver functions).

S. Smith, *Med. World*, 17/5/35 (Recent advances in legal medicine).

Harvey, " Luminescent Substances of Animals " (London : Chapman & Hall), 1935.

A. Stern and H. Molvig, *Z. Physikal Chem.*, 1935, **175**, 38 ; 1936, **176**, 209, 347 (Relationship between the structure and fluorescence spectra of porphyrins).

B. Aznar, *Ann. Méd. Légale Crim. Police Sci.*, 1935, **15**, 781 (Relative merits of infra-red and ultra-violet radiation for the examination of hair).

U. N. Brahmachari, *Nature*, 1936, **137**, 330 (Presidential address to the Indian Science Congress ; Science and Progress in Medicine).

P. A. Mattis, *Amer. Jour. Pharm.*, 1936, **61**, 706 (Fluorescence of constituents of the endocrine glands).

M. Déribéré, *Rév. Médicine*, 1937, No. 8, 469 (General).

Ch. Dhéré, " La Fluorescence en Biochimie " (Paris : Presses Universitaires de France), 1937 (Special reference is made to the author's work in porphyrins, urobilins, etc.).

H. Bierry and B. Gouzon, *Documentation Sci.*, 1936, **5**, 309 ; 1937, **6**, 17 ; *Compt. rend.*, 21/3/38 (Applications of fluorescence spectroscopy to medicine ; in particular to flavins, oestrogenic hormones, etc.).

B. Blasius, *Koll. Zeit.*, 1938, **84**, 74 (Dyestuffs suitable for histological fluorescence microscopy).

G. A. K. v.d. Borne, *Ned. Tijd. Geneeskunde*, 1938, **82**, 11, 1808 (Fluorescence of urine due to indoxyl compounds).

H. Minibeck, *Biochem. Z.*, 1938, **297**, 29, 40, 214 (Determination of the bile acids in body fluids by means of the fluorescence photometer).

A. Boutaric and J. Bouchard, *Compt. rend. Soc. Biol.*, 1935, **120**, 293 (No inhibition of the fluorescence of uranine is produced by sera from mice having cancer).

A. Hadjioloff, *ibid.*, 1938, **128**, 1096 (Fluorescence analysis of tissue cultures).

A. Hirt, J. Ansorge and H. Markstrahler, *Z. Ges. Anat.*, 1938, **109**, 1 (Fluorescence microscopy of the livers of the frog and rat).

C. Dhéré and V. Castelli, *Compt. rend.*, 1938, **206**, 2003 (Synthetic flavins).

M. Fontaine and R. G. Busnel, *ibid.*, 1938, **206**, 1679 (Flavins and fluorescent substances in skin and scales of fish).

T. Tadokoro, *Jour. Agric. Chem. Soc. Japan*, 1938, **14**, 210 (Fluorescent acetylflavin and like substances in plants and animals).

A. Drilhon and R. G. Busnel, *Compt. rend.*, 1938, **207**, 92 (Flavins in Lepidoptera).

J. Thomas, *Bull. Soc. Chim. Biol.*, 1938, **20**, 471 (Porphyrins in biology and pathology).

CHAPTER XII.

MINERALS AND GEMS.

A NUMBER of workers have examined minerals and precious stones under the lamp, and from their results (summarised in Table 15) it must be concluded that the behaviour of these substances in ultra-violet light depends to a very great extent on the place of origin. The colour of the gem itself has often an influence on that of the fluorescence, and the presence of small traces of some compounds often modifies the fluorescence of a mineral, or causes fluorescence to appear where it would not otherwise be in evidence. F. V. Lutati [13] considers that the fluorescence of minerals is not sufficiently characteristic to serve as a means of identification except in certain cases such as calcite (p. 267), celestite, fluorite (p. 269), hydrozincite, rubies and zircon, but that the variation in fluorescence of several samples of the same mineral from different localities may have some uses for the sorting of different samples for collections. The use of ultra-violet light in museum work is dealt with by S. G. Gordon [12] and in Chapter XIII, and various sources of the light are compared by Barrett.[46]

The examination of minerals in the form of a powder using the fluorescence microscope, and in thin leaflets, has been carried out by E. Engelhardt [3] and by H. Lehman,[2] and the results are also given in Table 15.

MINERALS.

In 1903 G. F. Kunz and C. Baskerville [1, 21] examined a large collection of minerals and gems containing over 13,000 specimens by means of ultra-violet light, X-rays and the γ-rays from radium, and a brief summary of their results with ultra-violet rays follows.

Of the *rare earth oxides* examined only those of thorium and zirconium showed any fluorescence in ultra-violet light, although,

as Haberlandt has shown (p. 269), certain rare earths may modify the fluorescence of the fluorites. The natural carbonates, witherite, strontianite and barytocalcite showed strong fluorescence; of the samples of cerussite examined one only (from Phœnixville) responded. Glauberite from Chili showed no fluorescence, but those from Borax Lake, in California, and from Laramie glowed vividly. With pectolite a fluorescence was obtained even from specimens that had almost completely changed over to steatite, and a fluorescence was also obtained with wollastonite.[17] F. G. Wick [65] has recorded numerous data concerning triboluminescence, thermoluminescence and tribothermoluminescence (see Chapter I), notably of fluorites, willemite and rare earth minerals (see also Chapter VIII). These effects may be induced by exposure to X-rays. According to L. Royer,[66] eruptive rocks and certain rocks which contain orthoclase are weakly thermo-luminescent.

Calcium Minerals.—It is interesting to note that these same workers found the fluorescence of samples of gypsum from Sicily to be from two to five times as intense as that from samples from Bavaria and elsewhere, and one of us (J. G.) has also noted different shades of red and violet in samples (" mineral white " or " pearl hardening ") submitted for use in paper manufacture; it is not yet clear that these can be completely related to the source of the mineral.[47, 48] After exposure to radioactive radiations, calcium sulphate containing about 0·001 per cent. of thallium has a red fluorescence at low temperatures.[61]

W. Witteborg [26] and A. Josten,[27] however, examined calcite minerals rather from the point of view of faults in crystal structure, and the former has claimed to be able to demonstrate comparative ages in this way. Similarly, Josten found that ultra-violet light renders the " sundial " structure much more apparent, and he considers (with Iwase [58]) that the fluorescence may be due to a foreign substance which is segregated in the direction of crystal growth. S. Imori and his co-workers [40] found that monoclinic perthitic felspars found as constituents of pegmatite had an intense thermoluminescence. Calcites were also studied by A. Köhler and H. Leitmeier,[41] and by E. Iwase and T. Kuronuma.[59]

Marble is treated more fully under Museum Work (p. 285), but mention may also be made here of the work of H. Haberlandt,[28] since he also used ultra-violet light to study crystal structure and faults. Chalk is also dealt with elsewhere (see

J. Grant, p. 340), but W. E. Naylor and A. Surfleet [53] show how prepared and precipitated chalks (of Pharmacopœia quality) may be differentiated by their flesh-coloured and dull-violet fluorescence respectively.

M. Déribéré [60, 79] records that a very persistent fluorescence may be observed from some natural calcium compounds. Thus fragments of stalactites and stalagmites have been found to continue to fluoresce visibly for periods up to 7 seconds after removal of the exciting source. This may, in fact, be regarded as a form of phosphorescence (see p. 3, and V. Levshin and M. Alentzev [67]). The intensity of the fluorescence falls gradually during this period, but the shape of the curves is, as a rule, unaffected by the temperature, although in some cases the intensity falls off at the higher temperatures ; this decrease is often associated with the loss of occluded water. The presence of silica is without influence on the phenomenon, but traces of iron (*e.g.*, 0·5 to 1 per cent. as Fe_2O_3) have an inhibiting influence. The red-orange fluorescence of certain manganiferous calcites increases with the manganese carbonate content, reaching a maximum at 3·5 per cent., and disappearing at 17 per cent.[68] The presence of aragonite is not considered to be an important factor in determining the nature of the fluorescence.[71] The colour of the fluorescence of certain calcites changes when fresh surfaces are cut.[63]

Phosphatic minerals used as fertilisers are considered on page 90, but the work of H. Haberlandt [32, 33, 36] and his colleagues on apatite and other phosphates, scheelite and zircon, should be mentioned here because it has established the use of fluorescence spectra for the detection of rare earths. F. Mach and P. Lederle (see p. 90) found that most phosphates fluoresce (a notable exception is basic slag from the Thomas process), but that in many cases the fluorescent constituent can be extracted with alcohol ; since it appears to be greasy in nature, they attribute it to contamination during the milling process.

Micas, particularly of the kitzenbuchel type, were examined by H. Nieland,[31] who was able to demonstrate the presence of particles of haüynite (even in the pseudomorphic state), since these fluoresce vividly.

Autunite and another uranium mineral from Mitchell County, N.C., fluoresced with the typical uranium colour, although speci-

mens from other countries did not, and Photograph No. 28 (see p. 400) by W. M. Thornton and M. N. Lewis [55] show how autunite disseminated in Dakota porphyry may be rendered visible. There appear to be two minerals present in autunite, both of which fluoresce, but with an orange and lemon-yellow colour, respectively. A specimen of hyalite in a trachytic rock from Mexico fluoresced with an intense green colour, but no other sample of hyalite gave such a fluorescence.[54] Specimens of willemite from Franklin, N.J., but none of the others examined, had a yellow-green fluorescence (*cf.* Table 15). A. Estrafallaces [22] also gives data for a large number of minerals, and photographs published by W. M. Thornton and M. N. Lewis [55] show how a vein of willemite in franklinite may be rendered visible by means of its green fluorescence, if a filter is used which passes through this ray but cuts out the fluorescence due to other inclusions.

Fluorites.—Estrafallaces [22] also records the fact that fluorites from the same locality may fluoresce differently, but most of the pioneer work on these minerals is associated with the name of H. Haberlandt.[18, 32, 33] Haberlandt noted red zones or specks in the fluorescence of fluorites from various parts of Germany. In some cases this appeared only after heating and/or irradiation with β- or γ-rays; with others, however, such treatment was without effect. No spectral lines corresponding with rare earth elements could be detected in the former fluorites, and since the specimens showing the natural red fluorescence came from radioactive regions, it was concluded that this colour was derived from the same source as in the case of the artificially irradiated fluorites. M. Déribéré [56] points out that the violet photo-luminescence common among the fluorites, is not connected with their thermoluminescence, as it is modified by the inorganic colouring matters which usually occur in this mineral. Rare elements and bituminous impurities [57] may also account for the nature of the fluorescence observed, and K. Przibram [69] has proposed a theory which relates the effects observed in the former case with electronic transitions.

Experiments were also carried out [33] on the normal fluorescence of fluorites, which has been attributed in some cases to rare earths and bituminous impurities; the natural red "radiophotofluorescence" is attributed to radioactive inclusions, whilst bituminous matter produces a yellow-white colour.[43] W. de Groot [34] also had

previously attributed the band spectrum (4000 to 5000 Å.) observed to heavy metals in the colloidal state, rare earths being considered responsible for the line spectrum. Haberlandt and his colleagues [33, 42, 62] found, however, that rare earth lines were visible only when high concentrations of yttrium were present, and his experiments with synthetic fluorites (*i.e.*, calcium fluoride containing 0·1 per cent. of rare earths) showed that europium is probably responsible for the blue and ytterbium for the green component. S. Kreutz [35] has also examined the effects of low temperatures on the fluorescence of fluorites and willemite (see below).

K. Przibram [70] found that when europium oxalate is heated with calcium chloride, the resulting product had a fluorescence similar to that of certain fluorites, and he therefore suggests that fluorites contain traces of divalent europium. H. Haberlandt [72] confirmed the close connection between the rare earth content of fluorites on the one hand, and the fluorescence and thermoluminescence on the other.

Willemite.—Spencer [10, 11] also found that the behaviour of *willemite* varied according to the source (see Table 15), and although fluorescence is not a sure test as to the presence or otherwise of willemite, the method is used by the New Jersey Zinc Company for the examination of the tailings of the crushed ore. Willemite has also been the subject of spectroscopic examination by C. Parlache [14] and T. Liebisch,[4] who found that specimens from Rhodesia showed no fluorescence, whilst others had a dull green colour. That the fluorescence colour of the willemite is not related to the colouring of the crystal is shown by the fact that some colourless crystals gave a rich violet fluorescence, and that it is often the coloured specimens which show no fluorescence at all. Artificial willemite does not fluoresce when pure, but the addition of an alkali or of a trace of manganese induces the phenomenon.[49]

A pale green cleavage slab of willemite from Franklin Furnace, examined by L. J. Spencer (*loc. cit.*), gave a brilliant uranium-green fluorescence, followed by phosphorescence, but another sample from the same locality had pale green willemite grains embedded in a cleavage mass of white calcite, the resultant effect being a flesh-coloured dark red mass with a number of brilliant green fluorescent spots. Specimens from Altenberg

(Belgium), Broken Hill, Sable Antelope Mine, South-west Africa, and from Durango, Mexico, did not fluoresce. Some samples of the red radiating willemite, which form the base of the colourless crystals from Broken Hill, did, however, fluoresce, although only in patches, with a bright yellow colour. The significant conclusion to be drawn from these results is the dependence of the fluorescence of willemite on the content of its minor constituents, and it is unfortunate that no attempts have been made at a closer correlation between the colour of the fluorescence and the chemical analysis. Other zinc minerals from Broken Hill were also examined by Spencer and a variety of results obtained. Thus two specimens of calcium carbonate and smithsonite gave three different reactions (see Table 15).

An interesting specimen of granular *black blende* from Tsumeb, South-west Africa, was examined in the piece and showed the phenomenon of triboluminescence, *i.e.*, on abrasion it gave streaks of yellow sparks; under the lamp this sample glowed with a fiery yellow colour. Several workers have noted that exposure to heat, or to β- or γ-rays, induces fluorescence in certain minerals [18-20, 47, 48] (see p. 269).

The fluorescence properties of a number of minerals have also been examined by S. Kreutz,[29] L. Royer,[16] W. L. Brown,[24] and C. C. Pines,[25] and others.[38] Royer found that anglesite has a fluorescence similar to, though distinguishable from, that of galena, and it is also possible to differentiate anthracitic and graphitic from bituminous and carboniferous rocks. Neither opal nor the particular sample of willemite examined by Royer fluoresced immediately in ultra-violet light, but the former did so after exposure for a few minutes. Kreutz records the intensities and spectral lines of the fluorescence from fluorite, apatite, calcite and topaz, and attempts to relate them to the ages of the specimens. In his studies on the fluorescence of natural scheelite, wolframite and hübnerite, F. R. van Horn [30] came to the conclusion that the degree of weathering exerts a potent influence on the nature and intensity of the colours observed, and the presence of radioactive elements (*e.g.*, in scapolites [37] and in hyalites containing uranium [45]) may also produce variable results. Both W. O. Vanderburg [77] and O. F. Herzger [78] describe how fluorescence analysis can be of assistance in prospecting for scheelite ores.

TABLE 15.

FLUORESCENCE OF MINERALS

Mineral.	Source and Particulars.	Colour of Fluorescence.
Amber	(See p. 326)	Yellow-green to blue-white
Apatite	Various	Red or yellow [36]
Aragonite	Franklin Furnace, N.J.	Rose-red [1, 39]
Autunite	Mitchell County, N.C.	Typical uranium colour [1]
Black blende	S.W. Africa	Fiery yellow (tribo-luminescent) [10, 11]
Calcite (see p. 267)	Franklin Furnace, N.J.	Red to violet [1, 26, 27, 29]
Doucil	Synthetic	Dull purple
Fluorites	(See p. 269)	Red specks [18, 32]
Glauberite	Chili	None [1]
Hackmannite	Bancroft, Ontario	Blue fluorescence with salmon-coloured zones; blue thermo-luminescence [74]
Hemimorphite	—	Dull white [10, 11]
Hopeite	—	Pale yellow (patchy crystals) [10, 11]
Hyalite (trachytic)	Mexico	Intense green [1]
	Other sources	None [1, 54]
Hydrocarbons	Solid (in rocks)	Yellow [10, 11]
	Liquid	None [10, 11]
Hydrozincite	Algiers	Blue [1]
	Other sources	Bright white [10, 11]
Leucite	—	Red, white or orange [10, 11]
Leucophan	—	Red to violet [2, 3]
Nepheline	Basaltic	Red-brown [2, 3]
	Most sources	Red, white or orange [10, 11, 64]
Opal	Mexico and Baden	Sap-green [10, 11]
Scapolite [75]	(See p. 271 and under Wernerite)	—
Smithsonite	White coarse mammilated crystals	White, green tinge [10, 11]
	White, fine botryoidal rhombohedra	None [10, 11]
Sodalite	Most sources	Orange-red [2, 3, 10, 11, 64]
	Crystalline	Green-violet [2, 3]
Wernerite (Scapolite)	Canada	Yellow fluorescence and green thermo-luminescence [74, 75]
Willemite [73] (see p. 270)	Franklin Furnace, N.J.	Uranium green with pale green cleavage [8]
(in calcite)	Do.	Green spots, flesh-red ground [8]
(Yellow crystals)	Altenberg, Belgium	None
(Colourless crystals)	Broken Hill, N. Rhodesia	None
„ „	Sable Antelope, Rhodesia	None
(Yellow crystals)	Guchap, S.W. Africa	None
(Colourless crystals)	Peñoles, Mexico	None
(White or yellow radial aggregates)	Lusaka, Rhodesia	Dull, dark green
(Troostite)	Stirling Hill, N.J.	Dull, dark green
(Yellow rock)	Rhodesia	Purple streaks and yellow and blue spots

GEMS.

Some early work using the fluorescence microscope was carried out by A. v. Loehr,[7] but Spencer (*loc. cit.*) also examined a number of precious stones. *Opals* were found to vary in response (Table 15), and of the samples of zinc blende examined only those from Tsumeb and a pale coloured variety from Beaver County, Utah, showed any fluorescence. The fluorescence of Japanese hyalite (a form of opal) is probably due to uranium compounds or other radioactive minerals.[54]

H. Michel [5] and G. Riedl [8] introduced the use of colour filters for the observation of the fluorescence colours of precious stones, and thereby obtained a rough classification of the fluorescent light and also eliminated the colour of the gem itself. Much interesting information on the behaviour of gems in ultra-violet light, cathode rays and X-rays is given by the latter. *Diamonds* show various fluorescence colours or none at all according to the locality of their origin (see Photograph No. 27, p. 400). Colourless topaz, beryl quartz and zircon do not fluoresce.

A strong orange-yellow fluorescence is given by colourless or white natural *sapphires*, but artificial sapphires often have a pale reddish fluorescence, probably on account of the presence of chromium oxide. Yellow sapphires can be differentiated from all other stones by their beautiful orange-yellow colour with a reddish tinge. A much weaker but more yellow fluorescence is shown by *yellow topaz*, and these are the only yellow stones which fluoresce. Dark specimens of blue sapphires have practically no fluorescence, and the brighter forms show variations in intensity when viewed through the colour filters. The best differentiation is obtained with Ceylon and Montana sapphires, since the Australian and Indian stones react only weakly. The fluorescence of artificial blue sapphires is moderately strong and reddish-violet in colour. Artificial blue *spinel* fluoresces with a strong red colour, and a greenish to yellow fluorescence is usually shown by *blue topaz*.

Siam *rubies* may be differentiated from Burmese and synthetic rubies by their much weaker fluorescence. The pale purple variety of Ceylon rubies shows up strongly through a red filter, the fluorescence being often similar in intensity to that of Burmese rubies; they appear weak red and wine-red

through a blue glass. Bright, wine-red spinel fluoresces as strongly as Burmese rubies, the red variety fairly strongly, and the yellowish-red spinel about as strongly as Siamese rubies. Little or no fluorescence is observed with red zircon or with red beryl, garnets or tourmaline. The fluorescence spectrum of the ruby is similar to that of the sapphire.[52]

In a paper on the examination of precious stones, J. Bolman [50] refers to the application of fluorescence analysis but gives no examples, and in view of this and of the general lack of information on this branch of the subject, the following notes (by J. Grant [51]) may be of interest. They are selected from tests made on authentic samples representing 30 different types of precious and semi-precious stones, and the examples given below are intended to show how the method may be of use to distinguish stones which (except to the expert) appear alike.

A synthetic aquamarine is sometimes confused with genuine Burma sapphire, but may be distinguished by its dull pink fluorescence, the latter stone appearing dark purple or black, and in this respect indistinguishable from the synthetic sapphire.

White topaz, white zircon and diamond also appear alike in daylight, but have a dull purple, pale mustard-yellow and mauve to black fluorescence, respectively. The method fails to distinguish diamond from paste, but true diamonds show various shades of purple or mauve, presumably according to the method of cutting used (*cf.* Bolman, *loc. cit.*). Incidentally, it may be mentioned here, that O. Holstein [76] has found that white zircon changes in appearance after prolonged irradiation.

Opal ("whiteclyffe") has a fluorescence similar in colour to that of an egg-shell, the characteristic green fire and orange-yellow colour seen in daylight being invisible, and this easily distinguishes it from moss agate, which is grey with a blue streak.[51] Black opals have no fluorescence, and Mexican fire opals appear dull purple, but both of these stones are easily recognised by the unaided eye.

Chinese jade, amazonite, soudée emerald (beryl type) and chrysoprase also resemble one another, and the fluorescence effects though similar, are distinguishable, being dark green with bright streaks, dirty pale green, very dark green and pale opalescent green, respectively.

Finally, the brilliant, fiery deep-red fluorescence of the syn-

thetic ruby readily distinguishes it from the paler and more pink red of the genuine ruby.

Other fluorescence colours which may be of interest (although the stones concerned are easily recognised without their aid) are as follows[21]: Genuine and imitation amethyst, mauve to black and mustard-yellow, respectively; blue and yellow sapphire, pink and orange, respectively (*cf.* supra); red and blue zircon, dirty red and dirty yellow, respectively; yellow and white topaz, dirty yellow-brown and dull purple, respectively; peridot, garnet, carnelian and turquoise, dark brown-green, dull purple, dull purple and mouse-grey, respectively.

Pearls and artificial pearls have been examined by H. Michel[9] and also by J. A. A. Leroux.[6, 44] It is not so much the difference in the colour of the fluorescence that assists the differentiation between culture and artificial pearls, as the differences in transparency to ultra-violet light. Thus, if the pearl is placed on a photographic plate under the lamp, then in the case of the culture pearl the image obtained is of uniform intensity, whilst that from a genuine pearl shows dark and light patches. No actual fluorescence is shown by artificial pearls unless they contain uranium compounds as a colouring agent, since the other metallic compounds used as colourings do not fluoresce under the lamp.[15]

The differentiation of precious from imitation stones under the lamp has been tested by G. Riedl,[8] but this worker considers that the method, if more convenient, is not so sensitive as the use of X-rays or of cathode rays. Amber is discussed on page 326, and *jades* (*cf.* p. 285) have been examined by de Tizac.[23]

REFERENCES.

1 G. F. Kunz and C. Baskerville, *Chem. News*, 1904, 89, 1; *Electrochem. Met. Ind.*, 1906, p. 435.

2 H. Lehman, *Zeit. wiss. Mikros.*, 1914, 30, 417.

3 E. Engelhardt, "Luminescenzerscheinungen im ultravioletten Lichte" (Inaugural Dissertation. University of Jena, 1912).

4 T. Liebisch, *Sitzungsb. K. Pr. Akad. Wis. Berlin*, 1912, 229.

5 H. Michel, *Deut. Goldschmiede-Ztg.*, 1926, No. 16.

6 J. A. A. Leroux, E. Raub and K. W. Frohlich, *Zeit. Physik*, 1928, 48, 722.

7 A. v. Loehr, *Tschermaks. Min. Petr. Mitt.*, 1912, 31, 35.

8 G. Riedl, *Fachzeitschr. d. Wiener Jeweliere, Gold- und Silberschmiede*, 6 nos. 1-8.

9 H. Michel, "Die Künstlichen Edelsteine" (W. Diebener, Leipzig, 1926).

10 L. J. Spencer, *Amer. Mineralogist*, 1928, 14, 33.

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REFERENCES.

1 G. F. Kunz and C. Baskerville, *Chem. News*, 1904, **89**, 1; *Electrochem. Met. Ind.*, 1906, p. 435.

2 H. Lehman, *Zeit. wiss. Mikros.*, 1914, **30**, 417.

3 E. Engelhardt, "Luminescenzerscheinungen im ultravioletten Lichte" (Inaugural Dissertation. University of Jena, 1912).

4 T. Liebisch, *Sitzungsb. K. Pr. Akad. Wis. Berlin*, 1912, 229.

5 H. Michel, *Deut. Goldschmiede-Ztg.*, 1926, No. 16.

6 J. A. A. Leroux, E. Raub and K. W. Frohlich, *Zeit. Physik*, 1928, **48**, 722.

7 A. v. Loehr, *Tschermaks. Min. Petr. Mitt.*, 1912, **31**, 35.

8 G. Riedl, *Fachzeitschr. d. Wiener Jeweliere, Gold- und Silberschmiede*, 6 nos. 1-8.

9 H. Michel, "Die Künstlichen Edelsteine" (W. Diebener, Leipzig, 1926).

10 L. J. Spencer, *Amer. Mineralogist*, 1928, **14**, 33.

11 L. J. Spencer, *Mineral Mag.*, 1927, **21**, 388.

[12] S. G. Gordon, *Amer. Mineralogist*, 1929, **14**, 362.
[13] F. V. Lutati, *Ind. Chimica*, 1930, **5**, 15, 1222.
[14] C. Parlache, *Amer. Mineralogist*, 1927, **13**, 330.
[15] Anon., *Technik. Ind. Schweizer*, 1929, 44.
[16] L. Royer, *Bull. Soc. Franc. Min.*, 1930, **53**, 515 (through *Chem. Zentr.*, 1930, ii., 834).
[17] J. Hoffmann, *Sprechsaal*, 1932, **65**, 82 (through *Chem. Zentr.*, 1932, (i), 2004).
[18] H. Haberlandt, *Sitzber. Akad. Wiss. Wien. Math. Nat.*, 1932, **141**, 441.
[19] A. Köhler and H. Leitmeier, *ibid.*, July, 1932.
[20] S. Kreutz, *Acad. Sci. (Polish)*, July, 1932.
[21] J. Grant, *Sands, Clays and Minerals*, 1933, **1**, (3), 7.
[22] A. Estrafallaces, *L'Ind. Chimica*, 1932, **7**, 1360.
[23] A. de Tizac, *Ann. Inst. Actinol.*, Jan., 1928.
[24] W. L. Brown, *Univ. Toronto Studies, Geol.*, 1933, No. 35, 19.
[25] C. C. Pines, *Amer. Jour. Pharm.*, 1933, **105**, 563.
[26] W. Witteborg, *Zentr. Min. Geol. Paläont.*, 1932, **A**, 364.
[27] A. Josten, *ibid.*, 1930, **A**, 432.
[28] H. Haberlandt, *Stein Ind.*, 1931, 106.
[29] S. Kreutz, *Bull. Acad. Polonaise*, 1933, **A**, 215 ; 1934, **A**, 166.
[30] F. R. van Horn, *Amer. Mineralogist*, 1930, **15**, 461.
[31] H. Nieland, *Jahrbuch Min. Beilage*, 1931, **63A**, 83.
[32] H. Haberlandt, *Sitzber. Akad. Wiss. Wien*, 1933, **142**, 29.
[33] H. Haberlandt, B. Karlik and K. Przibram, *Nature*, 1934, **133**, 99 ; *Sitzber. Akad. Wiss. Wien.*, 11/1/34 ; 21/2/35.
[34] W. de Groot, *Physica*, 1923, **3**, 358 ; *Arch. Néerland. Sci.*, 1924, III, **7A**, 207.
[35] S. Kreutz, *Bull. Acad. Polonaise*, 1933, **A**, 573 ; 1935, **A**, 486 ; 1936, **A**, 501.
[36] A. Köhler and H. Haberlandt, *Chem. Erde*, 1934, **9**, 88.
[37] H. Haberlandt and A. Köhler, *ibid.*, 1934, **9**, 139.
[38] H. and K. Krejci-Graf, *Zeit. Krist.*, 1934, **88**, 260.
[39] V. Rosický, *Výroční Zpráva Maravski* (Brno), 1930, **6**, 58 (through *Mineral. Abst.*, **5**, 209).
[40] S. Imori, J. Yoshimura and S. Hata, *Bull. Inst. Chem. Res. Japan*, 1934, **13**, 1091.
[41] A. Köhler and H. Leitmeier, *Zentr. Min.*, 1933, **A**, 401.
[42] K. Przibram, *Nature*, 1935, **135**, 100.
[43] H. Haberlandt and others, *Vienna Acad. Sci.*, Dec. 1934 ; Feb. 1935 ; *Nature*, 1935, **135**, 320, 767.
[44] J. A. A. Leroux, *Deut. Goldschmiede-Ztg.*, 1930, **33**, 255.
[45] E. Iwase, *Sci. Papers Inst. Phys. Chem. Res. Tokyo*, 1935, **26**, 42.
[46] R. L. Barrett, *Amer. Min.*, 1934, **19**, 578.
[47] E. Iwase, *Sci. Papers Inst. Phys. Chem. Res. Tokyo*, 1935, **26**, 258 ; **27**, 1.
[48] H. Steinmetz, *Zentr. Min.*, 1934, **A**, 209.
[49] Marconi Wireless Telephone Co. Ltd., B.P. 471,190, 28/2/36.
[50] J. Bolman, *Chem. Weekblad*, 1937, **34**, 496.
[51] J. Grant, *Analyst*, 1937, **62**, 731 ; *Gemmology*, 1935, Dec. ; 1938, July.
[52] C. S. Venkateswaran, *Proc. Indian Acad. Sci.*, 1935, **2A**, 459.
[53] W. E. Naylor and A. Surfleet, *Pharm. J.*, 1936, **136**, 261.
[54] E. Iwase, *Bull. Chem. Soc. Japan*, 1936, **11**, 377.
[55] W. M. Thornton, Jr. and M. N. Lewis, *Bull. Soc. Franc. Min.*, 1934, **57**, 268.
[56] M. Déribéré, *Ann. Chim. Anal.*, 1936, **18**, 117 ; *Ann. Soc. Geol. Belg.*, 1937, **60**, 377.

[57] H. Haberlandt, *Sitzungsber. Akad. Wiss. Wien*, 1934, IIA, **143**, 591.
[58] E. Iwase, *Bull. Chem. Soc. Japan*, 1936, **11**, 475.
[59] E. Iwase and T. Kuronuma, *ibid.*, 1936, **11**, 513, 523, 528.
[60] M. Déribéré, *Compt. rend.*, 1938, **207**, 222; *Ann. Chim. Anal.*, 1938, **20**, 206.
[61] K. Przibram, *Nature*, 1937, **139**, 329.
[62] K. Przibram, *Sitzungsber. Akad. Wiss. Wien*, 1935, IIA, **144**, 141; H. Haberlandt, B. Karlik and K. Przibram, *ibid.*, 1935, IIA, **144**, 77, 135.
[63] K. Obernauer, *Zentr. Min.*, 1935, A, 357.
[64] L. L. Smith, *Amer. Min.*, 1937, **22**, 304.
[65] F. G. Wick, *Nature*, 1937, **139**, 603; *J. Opt. Soc. Amer.*, 1935, **25**, 57; 1937, **27**, 275.
[66] L. Royer, *Compt. rend.*, 1937, **204**, 602.
[67] V. Levshin and M. Alentzev, *Compt. rend. Acad. Sci. U.R.S.S.*, 1935, **2**, 541.
[68] W. L. Brown, *Univ. Toronto Studies, Geol. Ser.*, 1934, No. 36, 45; 1937, No. 40, 155; through *Brit. Chem. Abst. A*, 1936, 185.
[69] K. Przibram, *Nature*, 1938, **141**, 970.
[70] K. Przibram, *ibid.*, 1938, **141**, 1113.
[71] S. von Gliszczynski and E. Stoicovici, *Z. Krist.*, 1934, **88**, 260; 1938, **98**, 344; through *Brit. Chem. Abst. A*, 1938, (i), 421.
[72] H. Haberlandt, *Sitzungsber. Akad. Wiss. Wien*, 1935, IIA, **144**, 663.
[73] M. Déribéré, *Ann. Soc. Géol. Belg.*, 1938, **64B**, 104.
[74] M. Déribéré, *ibid.*, 1938, **61B**, 175.
[75] E. Iwase, *Sci. Papers Inst. Phys. Chem. Res. Tokyo*, 1937, **33**, 299.
[76] O. Holstein, *Deuts. Goldschmiede-Ztg.*, 1936, **39**, 150.
[77] W. O. Vanderburg, *Bur. Mines, Information Circ.*, 1935, No. 6873.
[78] O. F. Herzger, *Calif. Jour. Mus. and Geol.*, 1938, **34**, 331.
[79] M. Déribéré, *Ann. Soc. Géol. Belg.*, 1938, **62**, 156.

ADDITIONAL REFERENCES.

R. L. Barrett, *Amer. Mineral.*, 1934, **19**, 578 (Comparison of sources of ultra-violet light for the examination of minerals).

A. Köhler and H. Leitmeier, *Zentr. Min.*, 1934, A, 364 (Fluorescence data for natural sulphates at various temperatures).

O. Glasser and I. E. Beasley, *Phys. Rev.*, 1935, **47**, 570, 789 (Effect of exposure to light on the fluorescence excited in mineral substances by exposure to X- or γ-radiations).

L. J. Spencer, "A Key to Precious Stones," 1936.

S. Iomori, *Sci. Papers Inst. Phys. Chem. Res. Tokyo*, 1936, **29**, 79 (Photoluminescence of felspar).

J. Grant, *Rock Products*, 1937, **40**, 50 (Examination of rock products and allied materials).

L. Royer, *Compt. rend.*, 1937, **204**, 22/2/37 (Thermoluminescence of volcanic rocks from Algeria).

H. Carlsohn and G. Muller, *Ber.*, 1938, **71**, 863 (Differentiation of natural and activated earths).

E. S. C. Smith and W. H. Parsons, *Amer. Mineral.*, 1938, **23**, 513 (Behaviour of forty-three minerals in ultra-violet light).

A. Quinn, *Amer. Mineral.*, 1935, **20**, 466 (Fluorescence analysis in petrography).

L. M. Borgström, *Bull. comm. géol. Finlande*, 1936, **115**, 349; *Neu. Jahrb. Min. Geol.*, 1938, 404 (Fluorescence of Finnish minerals).

CHAPTER XIII.

MUSEUM WORK.

A NUMBER of uses for filtered ultra-violet light are provided by museum work,[50] since a distinction between spurious and genuine specimens can often be made, especially in the case of documents, stamps and paintings. Documents or books, either forged, restored, or altered in places, often show variations in fluorescence at places where the original has been altered. Written matter which has become faint in the passage of time often shows up clearly under the lamp,[51, 52] and these aspects of the subject are treated further in Chapter X, on Legal Work; Chapters XVI (Paper) and XVIII (Textiles) should also be consulted.

Documents and Inks.—Kögel[1] has examined *palimpsests* under the lamp and has brought to light a valuable source of historical information. Palimpsests are old parchments from which the monks erased the original writings in order to obtain fresh writing material. They carried out the erasures in many cases by rubbing with sand, but small particles of the ink remained fixed on the fibres of the parchment. Photographs of the irradiated palimpsest in ultra-violet light often enable the original script to be seen under the newer writing (Photograph No. 4, facing p. 400). Some of these palimpsests have been cleaned several times, so that the photographic reproduction often shows three or even more sets of writing. A case in point is a Latin breviary which L. Bendikson[59] found to consist of 200 palimpsests from 8 different manuscripts; and another interesting example (investigated by W. R. Mansfield[61]) refers to a parchment which was taken from the Sultan's palace at Constantinople in 1922; this was believed to be a letter from an English king to the Sultan. Although the writing had been washed off so that the skin could be re-used, it was possible to show in ultra-violet light that the letter was actually from Louis XVIII of France, and concerned the appointment of his ambassador.

The applications of fluorescence analysis to library work in general, and in particular in connection with the dating and preservation of books and documents is dealt with elsewhere in detail by J. Grant.[55] It is pointed out that in many cases, *faded writing* may be read by direct observation in ultra-violet light, and the Peniarth MS. (Photograph No. 26, see p. 400) is an example of this nature. On the other hand, indirect methods of rendering faded writing visible must sometimes be used, as the dirt may obscure the fluorescence. Thus, J. Grant (*loc. cit*) has used a mixture of vaseline and mineral oil in equal proportions, which is smeared on the underside of the paper in question. This penetrates the paper more rapidly at the point where no writing has been, and since it is vividly fluorescent, it shows up the writing in dark outline against a glowing background. R. B. Haselden [56] has suggested a similar device, a solution of anthracene in alcohol being brushed on to the reverse side of the paper to that on which the writing occurs. The alcohol is allowed to evaporate and the paper is placed in ultra-violet light, when the portions where the writing has been remain dark against the glow of the remainder of the paper, owing to their masking effect on the fluorescence of the anthracene. Advantages of this method are that the contrast is less transient than that obtained by the oil test, and there is less chance of defacing the specimen. Charred or partly-burned documents may also sometimes be deciphered by methods of the above kind. The introduction of methods of photography of invisible fluorescence (see p. 69) has also proved of great service in work of this kind.

The *Codex Sinaiticus*, which was purchased from the Russian Government in 1933 and examined at the British Museum, also yielded some interesting secrets as the result of fluorescence analysis.[57] One example is the concluding verse of St. John's Gospel, which has long been suspect on account of the differences in vocabulary, style, ink, etc., which distinguish it from the remainder of the *Codex*. The ultra-violet lamp has now revealed traces of half-effaced writing, showing that originally the scribe stopped at the last verse but one and finished off with a tail-piece, but that subsequently he changed his mind and erased the tail-piece from the vellum and added the last verse and a new tail-piece.

Many chemicals used for restoration purposes, such as the

removal of "foxing" marks, leave traces which are visible in ultra-violet light, and many marginal rulings, watermarks and collectors' marks also appear. The fluorescence of the paper itself, as shown on page 226, is also some indication of age and composition, especially as old paper does not contain fluorescent sulphides and the fluorescence of rag differs from that of wood-pulp.

Different *inks*, also, vary often in their appearance in ultra-violet light, although the differences are not so great as has sometimes been suggested (*cf.* p. 228). Inks of relatively recent periods, which contain reagents such as gallic acid, are, as a rule, inert under the lamp.[2] Manly and Rickert have investigated names and markings scribbled on Chaucer manuscripts and have succeeded in reading a number of them which do not respond to other methods of examination, and the fraudulency of a number of other doubtful markings has been confirmed. Laemmel[3] has examined the records of Galileo's trial by this method, and has shown that they are perfectly genuine and have not been altered in any way. *Invisible Inks* are discussed in Chapter X and elsewhere, but they are also of interest in connection with museum work, because they have frequently been used in the past for secret formulas and recipes and in diplomatic correspondence. Thus, Haselden[56] cites the correspondence of an ambassador from Baden at the English court, which yielded information of great interest when examined in this way.

C. Ainsworth Mitchell[4] has shown by experiments on a number of inks of various makes and ages, that although some of them may be differentiated under the lamp, a number appear the same, even if they are obviously different when viewed in daylight. Pencil erasures can be seen with ease in certain cases (see p. 226), the rays being reflected by the tiny particles of graphite buried in the paper. O. Mezger and H. Lagenbruch[5] have investigated the superimposition of pencil and pen writing. They came to the conclusion that only when definite inhibition of the reflection is apparent is there justification for assuming that the pen writing has been superimposed on the pencil, *i.e.*, that the graphite is under the ink. However, the existence of reflection does not necessarily prove the reverse as the graphite may be so fine and dense that the ink has run over the edges of the line, or on to the paper. As may be expected, the degree

of sizing of the paper affects the result, and with more absorbent papers it is less likely that the graphite will be covered. The writing should therefore be photographed at various angles to the direction of the strokes, as the ultra-violet light may be reflected in one direction only (see also Chapter X).

Philately.—E. Müller,[6] A. J. Sefi,[7] E. Locard,[38] G. Richter [8] and O. Stiner [9] have examined postage stamps under the lamp, and have thereby opened up a field of investigation in philately which promises good results. Its success depends partly on the fact that for a given stamp any addition of colour or other matter differing in composition from the original can be detected by the difference in the fluorescence between the original groundwork and the added substance. Hence ink or pencil erasures can be detected, and repairs carried out with materials differing in chemical compositions from those used in the original often become visible. The mucilage on the back of the stamp often shows a fluorescence which varies in colour and intensity according to the origin of the stamp.

Mr. Percy Seiffert (President of the Royal Philatelical Society) has confirmed to us the great usefulness of the method for general philatelic work. He kindly brought to our notice an interesting instance involving a very valuable Ceylon issue, the voided corners of which were frequently cut off by users who apparently thought that the stamps then looked rather more attractive on the envelope. Such clipped specimens are now, of course, much less in value, and it is not unknown for the corners to be replaced with the object of making a fraudulent sale. Ultra-violet light, however, renders such repairs visible, not only by reason of the different paper used, but also by showing up the join.

In addition the lamp may be employed to detect reprints and forgeries, since the fluorescence of the suspected specimen will differ from that of a genuine stamp, unless, of course, the materials are identical. In the case of a Holstein 1¼ sch. stamp of 1864 examined by Müller, for example, the original remained quite dull under the lamp, the paper and the colouring of the stamp appearing almost black; in a forgery of the same issue the paper remained white and the colouring appeared as a brilliant blue. Two issues of the same stamp may also often be differentiated under the lamp, but in general the greater the number of issues, the harder is the differentiation. A German

10 pfennig " Deutschland " stamp containing eosin and carmine fluoresces in orange and green, whilst the German " Germania " stamp paper of the pre-war issues fluoresces white, the war issue appears grey, and the post-war issue has a mauve fluorescence.

The lamp may also be used to distinguish between forgeries of over-prints from the genuine issue, but only in cases where these are in colour, as black inks seldom respond to ultra-violet light. The test is particularly suitable when the colour has been imitated chemically; a Baden No. 1 issue, for example, appeared dark under the lamp, whilst an imitation obtained by colouring a No. 5 issue was light brown in colour. Another example is the 6 Kreuser black on green, and the 9 Kreuser black on heliotrope, both of which showed a dark brown colour, although an imitation appeared bright green. W. R. Mansfield [45] has published a photograph of the invisible fluorescence of a forged Indian stamp produced by short-wave ultra-violet light. The details of this case are interesting, because the stamp was a 4-anna 1854 issue, with an inverted frame, and if genuine it would have been of great value. The results show that a normal 4-anna stamp had been photographed, and a life-size stereo block prepared, the head being cut out of the frame on the block. The frame of the original block was then erased, and replaced by an inverted frame produced by printing with cut-out stereo. Ultra-violet light, however, revealed that the stereo was out of register by about 0·01 inch, and this provided the clue to the method of forgery used.

W. H. S. Cheavin and B. D. H. Watters [10] have made the interesting discovery that if cancelled stamps, bearing a postmark, are placed in contact with unused stamps, the latter show under the lamp a reversed cancellation mark, which is invisible in ordinary light. As a result of further experiments it was found that, on placing an unused stamp in a wallet that had previously held a number of postmarked stamps, a jumbled mass of cancellation marks was revealed by ultra-violet light. The thin sheets of transparent paper placed between the pages of a stamp album also receive the impression of the cancellation marks on used stamps in contact with it, and, furthermore, the marks can be transferred through these sheets from one stamp to another. Eradicated cancellation marks are also rendered visible.[53]

Paintings.—Recently, paintings, Japanese prints and other works of art have been examined in ultra-violet light. A. P. Laurie [11] uses the method for routine purposes, and he notes that *repairs*, *forged signatures* and *over-paintings* can often be seen distinctly, although the method is not infallible, especially so far as the identification of pigments is concerned. A. Maché [12] quotes the case of a picture, attributed to Pissaro, which showed under the lamp the fluorescent outline of a signature which had been removed before that of Pissaro was substituted. The method is also of use to the picture-cleaner and restorer, as it enables him to follow the effects of his work, particularly when he desires to ascertain how much varnish is present.

R. Gassul [13] has also used the method, and Bayle examined a Reubens picture the signature of which was questioned. The strokes of the name showed plainly in ultra-violet light, but were very indistinct in daylight. Further work on the over-painting and renewing of works of art has been carried out by R. Maurer,[15] while H. Rinnebach [16] also mentions a specific case of the detection of the falsification of a signature on a very tarnished picture, and other similar uses for the lamp. De Cellérier [30] of the Musée du Louvre examined a number of pictures for retouching and found that one or two minor paintings, thought to be genuine, are only copies. He considers that the actual working-methods of the artist can be investigated by means of the lamp. *Varnishes* fluoresce, often to such an extent as to obscure the fluorescence of the pigments under them, and allowance must be made accordingly.[37]

Other instances of similar applications of the method [47-49] are the examination by E. Botolfsen [41] of a Mona Lisa attributed to Bernado Luini (1525), which indicated that it was painted between 1615 and 1660; of the "Wisternitz" Venus by L. Franz [42]; of artists' signatures by F. Hellwag [43] and W. Frenzel [46] (who examined the suspected signature of the "Bautzen" Venus); and J. G. Goulinat [44] cites three further cases in which the method was used in conjunction with X-ray and microchemical tests.

Pigments show various fluorescence colours (see also Paints, pp. 329 and 330, and Photograph No. 19, p. 400). Zinc-white has a light chrome-yellow fluorescence, whilst white lead and titanium white fluoresce white and violet respectively. Of the yellow colours, only orpiment has a bright yellow fluorescence,

since cadmium or chromes have either a greenish or a brownish-black appearance. Organic dyes and imitation red lead are readily recognised if compared with genuine samples of the substance concerned. M. J. Schoen and J. Rinse [14] have shown that a number of pigments of the same composition, but from different sources, vary in fluorescence, so that it should be possible to distinguish recent from early work.

Fossils and Remains.—These provide an example of an extremely interesting and valuable application of the lamp, for small amounts of *organic remains* incorporated in stones often fluoresce under the lamp, and photography in ultra-violet light will reveal a great wealth of detail. The structure of many fossils has been examined in this way, and imitations and hidden repairs are often clearly visible. The original sutures of *bones* which appear to be single, but which are really compound, are revealed. Fine photographs have been published by H. Miethe [17] (who first observed these phenomena), by A. Miethe and A. Born,[18] and also by Drevermann.[19] The fossils to be photographed should be perfectly free from dust, blemishes and finger-prints, all of which appear in the finished picture, and a filter consisting of a thin cell with parallel sides, 1 cm. in thickness, and containing a 1 per cent. solution of cerium ammonium nitrate, should be placed between the camera and the object. This is necessary in order to absorb any ultra-violet light reflected from the object which would fog the photograph (see p. 72, and Photograph No. 5, facing p. 400, and E. Wagner [20, 21]). K. Portratz and H. Ziegenspeck [40] found the fluorescence microscope of value for the investigation of fossils in peat.

In some prehistoric dwellings at Wisternitz a mammoth's tusk, carved to depict a woman, was found, and it was questioned whether this specimen was genuine or not; L. Franz [22, 23] was able to show by means of the lamp that the specimen was the untouched work of a carver of the glacial period.

Van Ledden-Hulsebosch [24] has shown that burnt bones are no longer fluorescent, although old unburnt bones fluoresce brilliantly. In this way he was able to prove that certain of the bones found in a Dutch megalithic tomb owed their colour to age and had not been heated. Another paper of interest to archæological workers has been published by L. V. Dodds,[25] and K. Hörmann,[26] of Nuremberg, has indicated the use of the lamp

for the examination of bodies dried in prehistoric times in Bavaria; P. E. Spielmann [39] has applied the method to bitumen used for embalming.

The peculiar fluorescence of the *shells* of gastropods (*e.g.*, the families *Trochidæ* and *Turbinidæ*) appears to offer a useful means for the detection of the presence of certain pigments, even when these are overlaid with other colouring matters.[27]

Mineralogy.—The examination of real and artificial stones under the lamp has already been mentioned, and C. Baskerville [28] tested in this way a collection of over 13,000 specimens. Beautiful lighting effects are obtained by placing a collection of fluorescing minerals in a cabinet irradiated with ultra-violet light, and several museums have installed this device.[58] According to J. J. Rorimer [31] newly-cut *jade* is uniformly bright, whilst old specimens show definite signs of ageing by a change in the colour of the fluorescence similar to that of marble (*infra*). See also Minerals and Gems, p. 267, and L. Royer.[29]

Sculpture.—Old *marble* fluoresces differently from freshly-cut or old re-cut marble. The old marble appears a mottled white, or white with yellow and blue tones, whereas the surface of freshly-cut marble appears a uniformly intense purple. Before examination the surface of the old marble should be cleaned carefully with distilled water in order to remove organic or other matter which may fluoresce or otherwise confuse the results. G. M. A. Richter [32] has examined the statue of Diogenes in the Metropolitan Museum (New York), and concludes from the mottled appearance of the base and the lower portions that these only are of ancient origin, the upper portion being of more recent date.

Alabaster also shows similar ageing phenomena to marble, but it is more difficult to correlate the extent of the change with the period of workmanship. Very old and thin-walled objects in alabaster show ageing throughout the entire thickness, and in such cases there is a tendency for breaks to exhibit the same white fluorescence as the original surfaces. The lamp is, however, useful for the detection of modern restorations or forgeries in objects of alabaster. With objects made from *limestone* the same surface phenomena are observed on ageing as with marble, though they are not so easy to distinguish. The lamp will, however, detect additions or restorations made with plaster,

with ground stone in the form of cement, or with new portions cut from actual stone, owing to the differences in the fluorescence between the old and the new.

Ivories, in general, react like bone, but the lamp affords a means of distinguishing between old and newly-cut specimens and the relative degree of ageing can be estimated fairly closely. Newly-cut ivories are treated in many ways in order to make them appear old. Contact with manure, bitumen, the skin or tobacco juice, burning, and even giving the article to a turkey to swallow, are all used in order to simulate the appearance of old age, but ivories treated by any of these methods are readily detected under the lamp. *Bone* reacts similarly to ivory, and Rorimer (*loc. cit.*) considers that the lamp may be used to indicate the age of most objects carved in bone.

Woodwork usually has but little fluorescence when freshly cut, but the patina produced on ageing has a characteristic colour (usually whitish) in ultra-violet light, although this must be distinguished from that due to finishes such as waxes, varnishes, etc. Incidentally, fluorescence analysis has enabled oil varnishes on old *violins* to be distinguished from spirit varnishes, and since it has been found in this way that the former were always used on old "Strads," it has been possible to evolve an explanation on this basis of the improvement of tone of violins on ageing.

Ceramics.—Ultra-violet light is valuable for the comparison of imitation and genuine specimens of tiles, vases and other objects in china, glass or porcelain by means of the difference between the fluorescence of the body and the glaze (see also Chapter IV). Surface alterations may be readily detected, for metallic lustres are almost always non-fluorescent, whereas restorations, which are generally carried out in paint or plaster, fluoresce very brilliantly. Rorimer (*loc. cit.*) mentions the case of a fifteenth-century Italian bowl which had been so cleverly restored that the most careful examination failed to show the more recent additions; under the lamp the old sections were dark purple in colour, whereas the new portions fluoresced brilliantly.

Similar considerations apply to *glass*, since ageing produces decomposition and other changes which result in a fluorescence. Imitation of ancient glass by surface treatment is also often revealed in ultra-violet light.

Further papers of interest to those using ultra-violet light for museum work will be found in the references,[33-36] and in the chapters on Textiles (p. 361), Minerals and Gems, (p. 266), Legal Work (p. 223), and Paints and Varnishes (p. 324).

REFERENCES.

[1] R. Kögel, *Wilh. Knapp. Halle a. S. Arch. f. Kriminologie,* 71, 85 ; *Die Naturwiss.*, 1922, vol. 43 ; *Sitzungsber. Preuss.-Akad. Wiss.*, 1914, 37.

[2] L. V. Dodds, *Antiquity*, 1929, **3**, 219.

[3] R. Laemmel, *Arch. f. Geschichte d. Math. d. Naturwiss. u. d. Tech.*, 1928, **10**, 405.

[4] C. Ainsworth Mitchell, *Analyst*, 1930, **55**, 747.

[5] O. Mezger and H. Lagenbruch, *Chem. Ztg.*, 1929, **53**, 965, 985, 1006. A. J. Quirke, " Forged, Anonymous and Suspect Documents " (Routledge), 1930.

[6] E. Müller, *Die Postmarke*, 1926, 120.

[7] A. J. Sefi, *Phil. Jour. of Gr. Brit.*, 1929, **39**, 151 ; *Phil. Cong. Year Book*, 1929.

[8] G. Richter, *Das Postwertzeichen*, 1926, no. 1 ; *Köhler's Philatel. Magazin*, 1926, no. 4.

[9] O. Stiner, *Berner Briefmarken-Ztg.*, 1927, **19**, 147.

[10] W. H. S. Cheavin and B. D. H. Watters, *The London Philatelist*, 1930, **39**, 261.

[11] A. P. Laurie, *Analyst*, 1930, **55**, 162 ; 1934, **59**, 664 ; *Chem. and Ind.*, 1933, **52**, 329 ; " New Light on Old Masters," 1935 ; *Medico-Legal and Crim. Rev.*, 1936, 4, 208.

[12] A. Maché, " Expertise Scientifique des Documents et des Oeuvres d'Art " (P. Montel), Paris.

[13] R. Gassul, *Deut. med. Woch.*, 1928, **54**, 918.

[14] M. J. Schoen and J. Rinse, *Chem. Weekblad*, 1929, **26**, 321.

[15] R. Maurer, *Photo. Korrespondenz*, 1928, **64**, 200.

[16] H. Rinnebach, *Antiquitäten Rundschau*, 1930, **14**, 28.

[17] H. Miethe, *Photo. Korrespondenz*, 1927, **63**, 69.

[18] A. Miethe and A. Born, *Paläontol. Zeit.*, 1928, **9**, 343.

[19] F. Drevermann, *Natur. u. Mus.*, 1927, **57**, 193.

[20] E. Wagner, *Paläontol. Zeit.*, 1928, **10**, 298.

[21] E. Wagner, *ibid.*, 1928, **10**, 215.

[22] L. Franz, *Deut. Ztg. Bohem.*, 1930, Aug. 1.

[23] L. Franz, *Antiquity*, 1931, **5**, 493.

[24] C. J. van Ledden-Hulsebosch. See Legal Work (ref. nos. 2, 3, 16).

[25] L. V. Dodds, *Antiquity*, 1931, **5**, 235.

[26] K. Hörmann, *Schumacher Festschrift* (Mainz), 1930, 77.

[27] F. Querner, *Proc. Vien. Acad. Sci.*, April, 1929.

[28] G. F. Kunz and C. Baskerville. See Minerals and Gems (ref. no. 1).

[29] L. Royer, *Bull. Soc. Franc. Min.*, 1930, **53**, 515 (through *Chem. Zentr.*, 1931, ii, 834).

[30] F. de Cellérier, *Int. Illumination Congress, N.Y.*, 1928, **9**, 22.

[31] J. J. Rorimer, " Ultra-Violet Rays and their Use in the Examination of Works of Art " (New York, 1931).

[32] G. M. A. Richter, *Metropolitan Museum Studies*, 1929, **2**, (1), 29.

[33] J. J. Rorimer, *Bull. Met. Museum of Art*, 1929, **24**, (7), 185.
[34] E. Bayle and A. Maché, *L'Illustration*, 1929, No. 4516, 271, and No. 4517, 304.
[35] International Congress for Study of Scientific Methods applied to the Examination and Preservation of Works of Art, Rome, 1930; *Mouseion*, 1931, vols. **13** and **14**.
[36] Neuberger, " Echt oder Fälschung," Leipzig.
[37] A. Eibner, *Z. angew. Chem.*, 1932, **45**, 301 (Identification of paint media).
[38] E. Locard, *Rév. Intl. Criminalistique*, 1930.
[39] P. E. Spielmann, *Jour. Egypt. Archæol.*, Nov. 1932.
[40] K. Portratz and H. Ziegenspeck, *Blat. Unters. u. Forsch. Inst.*, 1932, **6**, 6.
[41] E. Botolfsen, *Tiddskr. Kjemi*, 1934, **14**, 8 (through *Brit. Chem. Abst. B.*, 1934, 333).
[42] L. Franz, *Sudeta*, 1930, **6**, 89.
[43] F. Hellwag, *Kunst. Wirt.*, 1928, **9**, No. 14.
[44] J. G. Goulinat, *Mouseion*, 1931, **15**, 47.
[45] W. R. Mansfield, *Phot. Jour.*, 1934, **74**, 120; 1935, **75**, 169.
[46] W. Frenzel, *Mannus*, 1931, **23**, 146.
[47] A. Eibner, *Mouseion*, 1931, **13-14**, 70.
[48] C. Brandmeyer, *Kunstaukt.*, 1930, **4**, 9.
[49] H. Rinnebach, *Tech. Mitt. f. Malerei*, 1931, **47**, 54, 64, 87; *Neue Folge*, 1931, **3**, 5.
[50] A. Bruning, *Mus. Jour.*, 1928, **28**, 189.
[51] G. C. Brock and R. W. Ditschbourn, *Brit. Jour. Phot.*, 1933, **80**, 737, 753.
[52] R. Mellet and M. A. Bischoff, *Compt. rend.*, 1925, **181**, 868.
[53] C. A. Mitchell, *Medico-Legal and Crim. Rev.*, 1935, **3**, 3.
[54] Anon, *Nature*, 1935, **136**, 362.
[55] J. Grant, " Books and Documents. Dating, Permanence and Preservation " (London: Grafton), 2nd Edtn. 1939; *Librarian*, 1936, **25**, 150; 1938, **28**, 146.
[56] R. B. Haselden, " Scientific Aids for the Study of Manuscripts " (Oxford: University Press), 1935.
[57] T. C. Skeat, *Daily Telegraph and Morning Post*, 11/1/38.
[58] S. G. Gordon, *Nature*, 1938, **141**, 213.
[59] L. Bendikson, *Library Jour.*, 1/1/36.
[60] F. I. G. Rawlins, " Physics and Chemistry of Paintings " (London: Royal Society of Arts), 1937.
[61] W. R. Mansfield, *Medico-Legal and Crim. Rev.*, 1939, **7**, 29.

CHAPTER XIV.

ORGANIC CHEMISTRY.

Fluorescence and Chemical and Physical Properties.

It is found that most organic compounds fluoresce in one or another condition and to an extent which depends, in many cases, on the state and purity of the compound. Furthermore, a series of compounds differing from one to another only slightly in composition may fluoresce differently, so that important theoretical results are obtainable by systematic research in this field. In the case of hydrocarbons with two benzene nuclei, the heavier the molecule the greater is the displacement of the fluorescence towards the visible region.[130] One of the first workers who attempted to correlate the fluorescence of organic compounds with their compositions was R. Robl,[1] but, as was shown by H. Kauffmann and A. Beisswenger,[2] the pH value of a solution and other factors may alter the colour of the fluorescence (see p. 310). A. Schontag and H. Fischer[15] have attributed the displacement of the fluorescence spectrum of methyl acridone with varying pH values to the action of the electric fields of the hydrogen ions. Anomalies in the fluorescence spectrum in solvents at very low temperatures are also considered, and a mechanism for fluorescence and phosphorescence is suggested. L. Pincussen[3] mentions the interesting fact that in some cases the wave-length of the fluorescence colour of a particular substance increases with an increase in the dielectric constant of the solvent, and his data for " dimethylnaphtheurhodine " in various solvents are reproduced in Table 16.

It is interesting to note that with anthracene, the fluorescence shifts towards the shorter wave-lengths as the di-electric constant is increased.

In general, substances which have a complex structure are the most likely to fluoresce. Efforts directed towards finding a

connection between structure and fluorescence have led to some useful but general rules, but too much reliance cannot be placed on these in attempts to predict whether a substance will fluoresce or not, as some fluorescent compounds of simple composition but containing no "fluorophors" are known. Complex substances fluoresce because the electrons responsible for the fluorescence are protected from deactivating influences (*e.g.*, collision with other molecules) by the outer portions of the molecule. Unactivated molecules, by collision with activated molecules of the same kind, exert a very powerful quenching effect, and in pure liquids this, combined with the absorption of the activating rays by the first layers of molecules on the surface, allows very

TABLE 16.

Solvent.	Fluorescence Colour.	Dielectric Constant of Solvent.
Ligroin	Green	1·86
Benzene	Greenish-yellow	2·26
Ether	Green-yellow	4·36
Diethyl oxalate	Yellow	4·95
Ethyl benzoate	Yellow	6·04
Pyridine	Yellow	8·08
Acetone	Orange-yellow	20·1
Ethyl alcohol	Orange	21·7
Methyl alcohol	Red-orange	32·5

little fluorescence to take place. By diluting the liquid with a solvent collisions between the activated and unactivated molecules are greatly reduced, and the fluorescence appears.

The work of E. Bayle and his co-workers [4] shows that the curves obtained when the wave-length of the fluorescence colour is plotted against its intensity provide a criterion of the purity of the substance concerned, since they consist of peaks which, as the impurities are removed, gradually rise to stable maxima. S. Dutt [5] found that quinine, resacetophenone, anthracene, methylacridine, eosin, anthranilic acid and dicyanoquinol become non-fluorescent after recrystallisation from 18 to 38 times, but that, with the exception of dicyanoquinol, they all recover on keeping in air for periods varying from several days to a few years, or on heating at the melting-point for a short time. The fluorescence

of fluorescein is not completely destroyed by exhaustive purification (59 crystallisations), and even then it is completely regenerated by heating at 200° C. for 10 minutes, or by passage of a current of air through an alkaline solution for 12 hours. The melting-points given by Dutt for these substances are a little higher than those usually accepted. K. Kalle [170] has made the interesting observation that when a solution of diacetyl in water is irradiated with ultra-violet light for a few seconds to a minute, depending on the concentration, a yellow-green fluorescence develops at several points and gradually spreads through the liquid. When kept in the dark the fluorescence fades, and can again be developed by exposure. This process may be repeated, but after ten repetitions the intensity of fluorescence has decreased to about one-third of its original strength. The viscosity of the solvent may also influence the colour or intensity of the fluorescence (*cf.* p. 381); for example, J. Chloupek [91] has shown that the fluorescence of eosin in glycerol solutions decreases on heating or on the addition of water, to an extent which is proportional to the change in viscosity.

Analysis.

E. Bayle and R. Fabre [6] have used fluorescence methods for the detection of some organic compounds in mixtures (see also Dhéré [145] on pyrones, glycosides, etc.). Sodium salicylate can be distinguished in admixture with talc or borax, and novocaine can be distinguished from cocaine or stovaine. They examined many organic substances and compared the intensities of their fluorescence, taking that of solid sodium salicylate as 20. Table 17 shows some of their results, and may be supplemented from M. Haitinger's bibliography of the subject.[221]

It is interesting to note that neither aspirin nor basic bismuth salicylate fluoresce, and Danckwortt [8] mentions that this applies also to basic copper salicylate. Dithio-salicylic acid fluoresces with a blue colour and the di-iodo salicylic acid has a green fluorescence (see also Costeanu and Cocosinschi [149]).

L. Ekhert [9] has suggested a general test for a number of organic substances in which a 10 per cent. solution of *p*-dimethyl aminobenzaldehyde in sulphuric acid is the reagent. One c.c. of concentrated sulphuric acid is covered with 1 c.c. of an

TABLE 17.

Substance.	Colour of Fluorescence.	Predominating Wave-length in Å.	Intensity.
Benzene	No fluorescence	—	0
Phenol	No fluorescence	—	0
Pyrogallol	Dark violet	—	1
Resorcinol	No fluorescence	—	0
Hydroquinone	Dark violet	—	0·5
Benzoic acid	Weak blue	—	3
Sodium benzoate	White	—	4
Lithium benzoate	White to violet	—	3
Mercury benzoate	Violet	—	2
Benzonaphthol	Green	—	8
Salicylic acid	Pale violet	4640	8
m-Hydroxy benzoic acid	Light violet	4660	6
p-Hydroxy benzoic acid	Light violet	4730	3
Sodium salicylate	Violet	4580	20
Potassium salicylate	Violet	4540	14
Lithium salicylate	Violet	4580	12
Magnesium salicylate	Violet-blue	4630	5
Mercury salicylate	Green	—	5
Bismuth salicylate	No fluorescence	—	0
Quinine salicylate	Pale violet	—	2
Eserine salicylate	Violet-blue	4670	4
Antipyrine salicylate	Violet-blue	4680	11
Methyl salicylate	Green	—	4
Ethyl salicylate	Green	—	4
Amyl salicylate	Violet-blue	—	4
Benzyl salicylate	Violet	—	4
Salol	Greenish-blue	4780	10
Salophen	Greenish-blue	4780	10
Betol	Blue	4630	8
Aspirin	No fluorescence	—	0
Coumarin	Violet-purple	—	4
Saccharin	White	—	3
Sulphanilic acid	Yellowish-grey	—	1
Novocaine	Blue-violet	4670	18
Sodium *m*-hydroxy benzoate	Dark violet	—	2·5
Sodium *p*-hydroxy benzoate	Violet-purple	—	3
Dihydroxy benzoic acid	Greenish-blue	4780	11
Sodium dihydroxy benzoate	Violet	—	2

alcoholic solution containing 0·01 grm. of the substance, and the reagent is then added. On shaking and examining under the lamp the fluorescence colours indicated in Table 18 are seen. H. Eichler[92] has shown that *resorufin* (oxyphenazone) has a strong yellow-red fluorescence which can be used to detect it in alkaline solutions. The fluorescence is destroyed when the solution is acidified, or reduced with sodium thiosulphate or ferrous

hydroxide, but not with sodium sulphite, arsenite or hydrosulphite or with formaldehyde.

TABLE 18.

Compound.	*Fluorescence Colour.*
Benzene.	Dark brownish-red.
Naphthalene.	Dark olive-brown.
Anthracene.	Greyish-blue.
Phenanthrene.	Greyish-blue.
Quinone.	Brownish bordeaux-red
Quinoline.	Brilliant blue.

H. Eichler [167] has grouped organic solvents according to their behaviour with Magdala Red. Salm [168] found that this dyestuff does not fluoresce at *p*H 2 but does at *p*H 4, while in alkaline solution it is non-fluorescent. Eichler, therefore, groups organic substances according as a solution of Magdala Red in them is fluorescent or otherwise, a third group being those compounds in which the dyestuffs is insoluble. Solutions of this dye in water and dilute acids are fluorescent at high temperatures, and this is explained by a change in the degree of dissociation of the solvent with temperature. The behaviour of solvents towards filter paper stained with an aqueous solution of Magdala Red is also specific. The dyestuff is non-fluorescent in solid solution in certain compounds containing phenolic or carboxyl groups, but when such solid solutions melt a strong yellow-red fluorescence is observed. Eichler [169] has devised a fluorescence thermoscope based on this observation (see p. 53).

P. W. Danckwortt [93] has applied the resorufin test (*cf.* p. 199) for the detection of nitrobenzene which, like *p*-nitraniline, phenol, α-nitroso-β-naphthol and *p*-nitrotoluene, gives a yellow-red fluorescent solution when heated with concentrated sulphuric acid and then made alkaline with caustic soda. The detection of α- and *β-naphthols* has been dealt with by T. Tokio and M. Ishii,[94] and to detect β-naphthol G. de Haas [95] adds an equal amount of glacial acetic and sulphuric acids when a green fluorescence is obtained in the presence of 1 part in 100,000. α-Naphthol, phenol, cresol, glycol and creosote gave negative results. M. Déribéré [16] has found that if a few mgrms. of β-naphthol are dissolved in strong sulphuric acid and heated on a water bath for some minutes, a violet-blue fluorescence appears, which cannot possibly be mistaken for that from α-naphthol. Commercial naphthols are not

fluorescent in acid or neutral solutions but fluoresce in alkaline media. Unlike the nitro-derivatives, naphthol-sulphonic acids are fluorescent (see p. 297). E. Tommila [96] has elaborated a test for *glyoxal* and *formaldehyde* in which naphthoresorcinol in hydrochloric acid is added to a solution of the sample in ethanol, when the presence of glyoxal (1 part in 10^{-5}) is shown by a blue-green fluorescence and that of formaldehyde (1 part in 5×10^{-5}) by a blue fluorescence. A test for glycerol is given on page 178 under Foods, and one for sucrose on page 177.

F. Feigl and co-workers [147] find that dicarboxylic acids the carboxyl groups of which are separated by not more than two carbon atoms, give dyestuffs of the fluorescein type if heated with a little freshly-sublimed resorcinol and sulphuric acid. The salts, esters, anhydrides, amides, imides and nitrites of these or peri-dicarboxylic acids behave similarly. The fluorescence colours are various shades of green, and the sensitiveness of the reaction in daylight varies from 2·5 to 50 γ (for tartaric acid); in ultra-violet light this could, no doubt, be greatly increased. By heating with formic and sulphuric acids hydroxy-1-2-dicarboxylic acids are converted to β-keto-carboxylic acids, which react in their enolic form with resorcinol and sulphuric acid to give umbelliferone compounds which have a blue fluorescence. H. J. Vlezenbeek (see p. 125) has found a similar reaction for compounds containing a *p*-aminophenol grouping. Other tests (which may, on occasion, prove useful in foodstuff analysis) are those due to E. Eegriur.[97] *Lactic acid* may be detected by adding to one drop of the solution a crystal of *o*-oxydiphenyl and 1 c.c. of 96 per cent. sulphuric acid and heating at 85° C. for one minute, when a blue fluorescence is developed in the presence of 0·01 mgrm.; the limiting sensitiveness is 1 part in 50,000 (heat for 15 minutes). *Iso*butyl aldehyde gives under these conditions a green fluorescence. For *pyroracemic acid* one drop of the solution is mixed with magnesium powder and concentrated sulphuric acid, lactic acid (*supra*) being formed; the sensitiveness of the test is 1 part in 16,000. If the test for *p*-oxydiphenyl (*supra*) is used the sensitiveness is raised to 1 part in 100,000. *Tartaric acid* may be detected by adding 1·0 c.c. of a fresh 0·05 per cent. $\beta\beta$-dinaphthol solution in 96 per cent. sulphuric acid to one drop of the tartaric acid solution, heating to 85° C. for 30 minutes, cooling and examining under the lamp,

when a green fluorescence is produced in the presence of 1 to 10 mgrms. of tartaric acid, the limit being 1 part in 50,000. This test is valuable as it gives positive results even in the presence of many similar acids. *Glycerol* is heated for 10 minutes on a water-bath in a covered tube containing bromine, the last of the bromine being then removed by heat (and by sodium sulphite if necessary). On addition of concentrated sulphuric acid and *m*-hydroxy benzoic acid, over 0·005 mgrm. of glycerol produces a green colour.[160] *Allyl alcohol* is first oxidised to dihydroxy-acetone and the test is then applied. F. Schutz [17] detects glycerin by heating the sample to 120° C. with a 1 per cent. solution of anthranone in concentrated sulphuric acid. A yellow coloration is produced, which gradually turns redder in shade as the temperature is increased, and finally remains stable at 170° to 175° C. Simultaneously, an orange fluorescence is produced which is specific for glycerin. The limiting sensitivity of this reaction is 5 parts in 10^6 and it is not affected by reducing agents.

The fluorescence of *acetone* (*cf.* ref. 75, p. 210) has been studied from the theoretical viewpoint by H. G. Crone and R. G. W. Norrish,[98] R. Titeica [100] has dealt with phenanthrene, and other tests for organic compounds occur under Foods.

Other references to fluorescence tests for organic compounds are given by C. Dhéré [145] and occur on pages 99, 122 and 249, and Preservatives, Oils and Sugars, etc., are discussed in Chapter VI, on Foods.

Fluorescence and Chemical Constitution.

An accumulation of ring systems or of quinonoidal bonds appears to produce an increase in the intensity, as is shown by the results of F. Kirchhoff [10] who has compared, by visual judgment, the strengths of the fluorescence of a number of cyclic compounds containing one or more rings with a carboxyl, or a phenolic group (Table 19).

R. Tomaschek [101] states that the radicals $-OH$, $-OCH_3$, $=CH_2$, $-NH_2$ and $-CN$ intensify the fluorescence and displace it towards the longer wave-lengths, whereas the radicals $=CO$ and $-COOH$ weaken the fluorescence. When the radicals $-CO$ and $-OH$ occur together they neutralise one another in effect. The presence of halogens in the molecule weakens the

intensity without altering the position of the main bands, and the presence of unsaturated radicals in the side-chain tends to intensify the fluorescence. Data on the relationship between the chemical structure, the fluorescence and absorption spectra of simple aromatic amines, aniline and diphenylamine produced by radiations of various wave-lengths have been reported by N. Prileshaeva and R. Tschubarov.[18]

TABLE 19.

Compound.	*Colour of Fluorescence.*
Benzene and homologues.	No visible fluorescence.
Anthracene.[220]	Strong blue.
Naphthalene.[220]	Weak bluish-violet.
Pentacene.[220]	Very weak dark blue.
Phenanthrene.	Strong bluish-green.
Retene.	Strong violet.
Fluorene.	Very strong violet.
Phenol.	Weak violet.
Hydroquinone.	Strong dark violet.
β-naphthol.	Strong dark violet.
Benzoic acid.	Bright violet.
Gallic acid.	Greenish-blue.
Salicylic acid.	Strong bluish-white.
Rosin oils.	Strong bluish-white.
Colophonium.	Strong bluish-white.
Camphor.	Weak brownish-violet.

The benzoxazole derivatives examined by F. Henrich and F. Braun[102] have yielded data connecting fluorescence and constitution. They find that, in general, the presence of an —OH group para to the nitrogen, and direct substitution of the hydrogen attached to the μ-carbon atom by an aryl group are essential for the development of fluorescence. The influence of methyl groups in the μ-phenyl-*p*-hydroxybenzoxazoles is also discussed.

The position of substituents is of some importance and A. J. Allen and his co-workers[103] find that when a radical moves from the α- to the β-position the position of the wave-length of maximum intensity also moves, the concentration, presence of impurities and the source of light having very little effect on the quality of the fluorescence. They were also able to distinguish between the free acids and the sodium salts (see below).

A. and L. Lumière and A. Seyewetz[104] have also shown the importance of the position of the substituent in their work on the fluorescence of photographic developers. They find that the

para derivatives, *e.g.*, of diamines, diphenols and aminophenols, fluoresce strongly and can be differentiated from the corresponding *ortho-* or *meta-* derivatives; thus, *e.g.*, metol can be differentiated from *p*-amino phenol. The colours are generally violet or green and are enhanced by the presence of 0·50 per cent. of sodium sulphite or carbonate.

The absorption spectra and fluorescence of the acid sulphates of quinine and of cinchonine, cinchonidine, hydrocupreine, hydroquinine, optochine, eucupine, vuzine, quinidine, quinicine and quininic acid in 0·9M sulphuric acid have been examined by L. J. Heidt and G. S. Forbes,[105] and further work on constitution in its relation to fluorescence is due to P. Swings.[106]

E. Canals and P. Peyrot[107] have examined pure compounds and found that fluorescence was shown by all the oxygen-containing substances tested, by cyclanes, cyclenes and their derivatives, but not by all hydrocarbons. The presence of a methyl group was found to diminish or even to suppress the fluorescence. They have also determined the relative intensities of the fluorescence of 8 alcohols, having spectra between 4400 and 4900 Å.[19]

J. Moir[108] considers (*a*) that no organic compound fluoresces unless it contains an aromatic ring; (*b*) the second important factor is the nature and arrangement of the substituents. One of these must be an auxochrome when only one aromatic ring is present, but if two rings are present then an auxochrome is unnecessary, and fluorescence appears when the two rings are joined in two places by two groups of almost any nature. Arrangement of the groups in the first ring is probably of less importance than their nature. Groups in the *ortho* and *para* positions increase the fluorescence considerably, but a faint fluorescence is known with substances having a *meta* position filled, *e.g.*, *m*-nitro dimethyl aniline. Should there be two *ortho* substituents then one should be an auxochrome and the other should contain an unsaturated link adjacent to the element nearest to the ring. R. Kuhn and F. Bär[109] have shown that several members of the quinoxaline series have a blue fluorescence. P. Karrer and H. Fritzsche[20] have investigated the relationship between the constitution of the flavins and their fluorescence. 8-Methyl and 6.8-dimethyl–9–β–hydroxyethyl *iso*alloxazine, in either water or acetone, shows a brownish-yellow fluorescence in ultra-violet light, but not in daylight, thus differing markedly from the

alloxazines. H. von Euler, K. M. Brandt and G. Neumüller [21] have plotted the *p*H-fluorescence curves of alloxazine–6.7-dimethoxy and 4.5-diamino–3–methyl uracil, *iso*barbituric acid, dioxindole and acridine both in the solid state and in solution. The fluorescence and absorption maxima of the simple coumarin derivatives have been obtained by W. Czapska-Oarkienwicz.[22]

The following table shows the fluorescence of various compounds in concentrated sulphuric acid :—

TABLE 20.

Xanthene.	Brilliant yellow-green.
Dihydroanthracene.	Blue-violet.
Dihydroacridine.	Brilliant green.
Dihydrophenazine.	Very weak grey-green.
Phenoxazine.	Green.
Xanthydrol.	Very faint blue.
Anthracene.	Practically nil.
Acridine.	Bright bluish-green.
Phenazine.	Weak grey-green.
Phenoxazonium salts.	Practically nil.
Anthraquinone.	Nil or very faint orange.
Acridone.	Brilliant electric blue.
Phenazine oxide.	Very faint orange.
Xanthone.	Bright violet.

G. Brooks [172] has examined the relationship between the chemical constitution and the fluorescence of laccol, moreacol and their drivatives and finds that these polyhydric phenols represent a new type of fluorescent compound in which the double bond of the C_{16} side chain is responsible for the fluorescence.

Salt Formation.—It is very interesting to notice that fluorescent salts are sometimes obtained from non-fluorescent organic acids or, when the acid is fluorescent the intensity may be increased by salt formation. This phenomenon is particularly marked with zinc salts ; thus zinc hydroxyquinoline sulphonate has a strong yellow fluorescence equal in intensity to that of uranium salts, but this is absent from potassium hydroxyquinoline sulphonate. J. Eisenbrand [11] mentions that 6-hydroxyquinoline and apoquinine show an enhanced fluorescence in the presence of some metallic salts, and that although 8-hydroxyquinoline itself fluoresces very weakly, its solid derivatives of zinc, magnesium, and cadmium are brilliantly fluorescent. A few drops of an alcoholic solution of 8-hydroxyquinoline added to an alcoholic solution of cadmium or zinc acetate, of magnesium, strontium,

thorium or lanthanum nitrate, or of the chlorides of calcium or lithium, give solutions which are strongly fluorescent. In these cases there is no precipitation of the metallic complex, and hence 8-hydroxyquinoline may be determined in quinosol, and a micro-method for the determination of zinc is also provided.

The effect of salt formation on the fluorescence of the substituted cinnamic acids has been studied by H. Ley and R. Dreinhöfer,[12] and the fluorescence of the sodium and potassium benzoylacetonates has been examined by F. G. Segitz.[13] A weak brownish-white fluorescence is shown by the colourless sodium salt if it is free from solvent, but the yellow sodium salt has a strong yellow fluorescence with a brownish tinge. The fluorescence and chemi-luminescence of some amino phthalic acid compounds have been studied by H. O. Albrecht,[14] and by K. Gleu and W. Petsch[161]; F. Henrich and F. Braun[102] discuss the relation of fluorescence to the structure of the benzoxazoles; and A. Cotton[162] has studied polarimetry in ultra-violet light.

J. Weiss[23] has shown that many fluorescent substances which absorb light in the visible region can be reduced by the action of visible light, in the presence of inorganic reducing agents, which quench the fluorescence (see p. 198). K. S. Gururaza Das[24] has advanced a theory of collision to account for the inhibition of the fluorescence of quinine sulphate and sodium fluorescein solutions by certain ions (*e.g.*, by Cl′, Br′, I′, etc.). For an explanation of the auto-inhibition of fluorescence, however, he finds it necessary to postulate a theory of polymerisation (see p. 382).

Chlorophyll.—The fluorescence of the chlorophylls and of the porphyrins has been studied by a number of workers,[186-188] but we shall deal here only with the fluorescence of the chlorophylls, since the porphyrins are discussed on page 249. The fire-red fluorescence of plant-chlorophyll has been known for a long while, and has been investigated recently by a number of workers, *e.g.*, by G. T. Dragone,[7, 33] who examined fruit juices, and L. Petri,[34, 35] who worked on the fluorescence of chlorophyll in the living tissue and on the detection of sulphur dioxide poisoning in plants.

Solutions of *plant-chlorophyll* show a fine red fluorescence,

but of its components the bluish-green chlorophyll-A has a characteristic red fluorescence, whilst that of the yellow chlorophyll-B is a milky white. G. T. Dragone (*loc. cit.*) considers that the fluorescence of chlorophyll in solution is only made possible by the separation, by means of the solvent, of the fluorescing compound from a non-fluorescent substance which may mask or inhibit the fluorescence of the former. It is well known that a number of commercial chlorophylls do not fluoresce, and K. Noack [36] explains this fact by suggesting that the chlorophyll exists in green leaves as an almost monomolecular layer or film on the protein of the chloroplasts. Colloidal chlorophyll is not fluorescent, nor is the absorbing body, and hence if by commercial treatment the two can be separated and the chlorophyll converted into the colloidal form, then no fluorescence will be observed.

In the plant chlorophyll is adsorbed on the chloroplasts, but if it is isolated and readsorbed on silica gel it shows on irradiation a fluorescence the intensity of which first rises to a maximum and then slowly falls almost to its initial value. The presence of oxygen quenches the fluorescence, which is restored when the oxygen is removed. Kautsky [173-174] finds that the intensity-time curves given by living leaves show four distinct phases and considers that, therefore, four distinct reactions occur in the assimilation cycle for oxygen.

Carbon dioxide is without effect on the fluorescence of the plant, but poisons, such as hydrogen cyanide, by stopping the respiration, cause an increase in the fluorescence. Kautsky and his co-workers [175, 178-180] therefore, consider that the assimilation of carbon dioxide is a surface phenomenon, and that, during the respiration of oxygen the light-activated chlorophyll activates the oxygen and so sets up a cyclic process. J. Franck, [176-179, 189-190] however, considers that an acceptor molecule-chlorophyll complex is formed, which absorbs the excitation energy after which a series of reactions take place, the first being the separation of a hydrogen atom from the chlorophyll. More experiments are obviously required before this subject can be considered closed (see also J. Weiss,[181] K. Weber [182] and O. Richter [183]).

A number of different forms of *commercial chlorophyll* are now on the market in which the magnesium present in the natural

chlorophyll is replaced by other metals such as copper, zinc, sodium, etc., in order to increase the tinctorial power and to render the commercial chlorophyll soluble in certain solvents. Such products are described as fat-, water- and spirit-soluble, pure chlorophyll (carotene free), and chlorophyll yellow (xanthophyll free). Xanthophyll and carotene have very little fluorescence, and A. Wilschke [37] and M. Tswett [38] have shown that the fluorescence is due, to some extent at least, to the presence of two other fluorescent components. *Solutions in alcohol* contain one component with fluorescence bands at 6700 to 6550 Å., and another with bands between 6550 and 6500 Å. In the living leaves the bands fall between 6860 and 6600 Å. for the first component, and between 6580 and 6550 Å. for the second. In this connection it may be mentioned that one of us (J. A. R.[110]) has examined a number of chlorophyll pills and in a number of cases the characteristic red fluorescence was not observed, a dull bluish colour being seen. Zickgraf [111] and U. Drehmann [112] have discussed and criticised the evaluation of chlorophyll under the lamp (*cf.* Drugs, p. 135). S. Hilpert and co-workers [114] have investigated the state of chlorophyll in the plant, and the connection between it and the carbon assimilation has been investigated by H. Kautsky and co-workers [115, 116] and by A. Stoll.[117] A. Dusseau [118] has attempted to correlate the fluorescence of chlorophyll solutions in alcohol extracted from wheat leaves with the nature of the species (see also J. Voss [166]), and other work on chlorophyll has been carried out by H. V. Knorr and V. M. Albers,[119, 184, 185] F. E. Lloyd [120] and others; the determination of purpurin using a Pulfrich refractometer has been studied by E. Vigliani,[121] and photoflavins have been examined by K. G. Stern and E. R. Holiday.[122]

The *hydrogenated derivative* of chlorophyll (phæophytin) also gives the red fluorescence characteristic of chlorophyll. When a solution of chlorophyll in alcohol is heated with small pieces of copper foil, the red fluorescence of the solution gradually decreases in intensity, and the same effect is observed when an alcoholic solution of mercuric chloride is used. The fluorescence disappears suddenly on warming with solutions of copper sulphate or lead acetate, but addition of zinc sulphate does not affect the fluorescence, and it is possible to evaporate the solution to 1 c.c. without observing any considerable change.

The *fluorescence spectra* of the chlorophylls and polychromoproteins both in solution and in the living algæ have been studied by C. Dhéré, M. Fontaine and co-workers.[39-44, 145] The pigments phycocyanine and phycœrythrin consist largely of proteins, and the size and nature and other physico-chemical properties of the molecular aggregates appear to be the chief factors governing the fluorescence. C. Dhéré [113] has further found that pyocyanine hydrochloride in aqueous solution dissociates into the free base without showing fluorescence, although solutions in chloroform have a green-yellow fluorescence. When the aqueous solution of the hydrochloride is cautiously reduced with sodium hydrosulphite in the presence of ammonia, or with zinc powder, magnesium or mercury, this fluorescence is developed.

Glucose added to a solution of the free base in caustic soda solution produces a red reduction-product which has a deep green fluorescence, and this method may be used as a test. Danckwortt has applied the fluorescence of chlorophyll to its detection in tinctures and extracts as mentioned on page 135, and further work on the fluorescence of chlorophyll has been carried out by K. Stern [45] and by J. Wlodek.[46] Since exposure to ultra-violet light destroys the fluorescence of chlorophyll, care should be taken with photographic work involving long exposures.

Perfumery Products.—Organic compounds used in perfumery have been examined by A. Müller [47] and by R. A. Morton,[151] and some of the results (Table 21) show that the predominating colours of the fluorescence are brown, violet and green.

P. Müller [90] noted that the normal clear violet colour of lavender oil becomes milky if more than 10 per cent. of substitute is present.

It will be seen that, in general, nitrogen compounds, lactones, phenols, ketones and aldehydes have a yellow or brown colour, whereas the other classes of compounds show green or violet as the predominating tint.

The investigation of a large number of the *essential oils*, tinctures, absolutes and synthetic perfumes, both pure and in alcoholic solution has been carried out by R. Escourrou,[48] who examined the substances in acid and alkaline solution. He considers that the fluorescence is, in many cases, useless as an exact criterion of purity, and in this he is in agreement with Müller *supra*; it is, however, a useful guide to identification.

TABLE 21.

Alcohols.	*Colour of the fluorescence.*
Absolute alcohol.	Dirty yellow.
Citronellol.	Weak brownish-green.
Rhodinol.	Weak violet with yellow tinge.
Benzyl alcohol.	Weak grey.
Phenyl ethyl alcohol.	Very weak violet.
Terpin hydrate.	Yellowish-brown.
Eugenol.	Dark chestnut-brown with milky tinge.
Esters.	
Diethyl phthalate.	Pale milky blue.
Benzyl acetate.	Bright violet.
Benzyl benzoate.	Grey.
Terpinyl acetate.	Greenish-brown.
Methyl anthranilate.	Bright milky bluish-green.
Methyl cinnamate.	Bright violet.
Linalyl acetate (pure).	Yellow-green.
Ethers.	
Iso-homoveratrol.	Milky-yellow
Nerolin.	Violet.
Diphenyl ether.	Violet.
Aldehydes.	
Anisaldehyde.	Clear brown.
Benzaldehyde	Clear brown with greenish tinge.
Heliotropin.	Bright yellowish-brown.
Cinnamic aldehyde.	Clear brown.
Hydrocinnamic aldehyde	Weak olive-green.
Vanillin.	Bright brown.
Ketones.	
Acetophenone.	Very weak brown.
Methyl acetophenone	Clear greyish-brown.
α-Ionone	Very weak, dirty dark green.
β-Ionone.	Clear greenish-brown.
Acids.	
Acetic acid.	Bright milky-brown.
Benzoic acid.	Violet.
Cinnamic acid.	Reddish-violet.
Phenylacetic acid.	Reddish-violet but darker than cinnamic acid
Phthalic anhydride.	Bright violet.
Lactones.	
Iso-capryl lactone.	Greyish-yellow.
Coumarin.	Clear violet.
Nitrogen Compounds.	
Indole.	Violet-blue.
Musk xylol.	Yellowish-brown.
Musk ketone.	Yellowish-brown.
Musk ambrette.	Yellowish-brown.

Citrus oils, which are almost non-fluorescent in the pure state, have a violet colour in a solvent containing an alcoholic hydroxyl group, and the colour of this fluorescence deepens as more solvent is added. Fractional distillation will not separate the substances

responsible for this violet fluorescence, and no fluorescence is developed in aldehydes, ketones, esters or solvents containing a phenolic hydroxyl group. According to R. Mellet and A. Bischoff,[49] bitter almond oil (clear yellow fluorescence), mirbane oil, oil of lemon (weak greenish-yellow) and lemon-grass oil, true and artificial musk, nitro- and amino- compounds can be differentiated, and they were also able to detect salicylic acid in 67 commercial pharmaceutical products.

E. Ekman and A. Samyschlayewa [50-52] have also investigated the fluorescence of perfumery chemicals from different sources, and note that, on the whole, such products show similar fluorescence colours although in some cases these are modified by small traces of impurities. Of the acids, acetic, formic and butyric acids give practically no fluorescence, and other acids examined showed colours similar to those obtained by Müller (p. 303). Chemically-pure alcohols show either a weak fluorescence or else none at all, and in such cases the colour generally has a violet tinge. Compounds whose fluorescence colour is other than violet are :—

Iso-amyl alcohol.	Light grey.
Nonyl alcohol.	Weak green.
Cinnamic alcohol.	Green.
Menthol (cryst.)	Greenish shimmer.
Borneol.	Dirty, weak, cloudy fluorescence.

In the aldehyde, acid, phenol and ketone groups several points of difference from Müller's work are also to be noticed. The ionones and various samples of methyl acetophenone, citral, anisaldehyde and pure benzaldehyde were found to give no fluorescence. One sample of eugenol gave a brown fluorescence, whilst two others had a violet colour, similar to that obtained with *iso*-eugenol. Lauric aldehyde appeared weak violet ; vanillin, dark brown ; phenacetaldehyde, light blue ; pure camphor, weak green ; and amorphous camphor, weak violet. Most esters fluoresced in shades of violet, or appeared milky-white. One sample of *iso*-amyl benzoate was brownish-white, but two other samples gave the usual violet fluorescence. These variations are undoubtedly due to the presence of impurities in small traces, some of which may be produced by oxidation.

C. P. Wimmer and M. H. Kennedy [53] also have examined

several hundred essential oils in rays of wave-lengths 3900 and 2400 Å. They state that none of the fluorescence colours observed were sufficiently characteristic to differentiate natural from synthetic essential oils, but they can, however, be used to detect adulteration. Terpinyl acetate, triacetin, etc., for example, can be detected by their fluorescence after fractional distillation of the sample, and addition of petitgrain oil to lavender oil, or the presence of petroleum oil or jelly in civet oil can readily be demonstrated. These results also enable the essential oils to be classified according to their chief components, *e.g.*, citral, cineole, eugenol, salicylates, anthranilates, etc.; moreover, lemon oils containing terpin may be distinguished from terpin-free, hand-pressed oil from citrus fruits. Petitgrain and neroli oils, eugenol, *iso*-eugenol, methyl eugenol and methyl *iso*-eugenol all give characteristic colours; pine oil has a lavender colour.[88]

The data of Wimmer and H. Goodman [123] for the fluorescence colours shown by essential oils and certain cosmetic ingredients are given below :—

Tragacanth.	Dull white-blue.
Calamine.	Grey-brown.
Lycopodium.	Dull green-brown.
Orris root.	Dark yellow.
Zinc stearate.	Pale brown.

E. Bottini [164] examined mandarin, orange, bergamot and lemon oils before and after distillation and in the natural state and in alcohol solution, and concluded that the fluorescence observed is the combined effect of a blue colour due to the constituents of high boiling-point (anthranilates), and a yellow colour due to pigments. A publication by Messrs. Schimmel [150] draws attention to possible errors that may arise from the use of the lamp for perfumery work.

T. Pavolini [54] has elaborated a fluorescence test for *sesame oil* which is specific and sensitive to 1 per cent. The oil is diluted with an equal volume of ether, and to 10 c.c. of the mixture are added 2 c.c. of a reagent made by mixing 80 grms. of 98 per cent. sulphuric acid, 10 grms. of 95 per cent. alcohol, and 10 grms. of distilled water; the acid layer then shows a striking green fluorescence.

J. Grant and H. Procter-Smith have examined authentic samples of methyl salicylate, oil of sweet birch (*Betula lenta*) and

oil of wintergreen (*Gaultheria procumbens*), kindly supplied by the late Mr. E. T. Brewis, F.I.C. The first gave a pale sea-blue colour, the two latter (which contained 97 to 99 per cent. of methyl salicylate) being bluish-white with a marked green tinge and pale golden-yellow, respectively. The differences are most marked, but it is possible that in drawing conclusions allowance should be made for any colour in the original oil, and for the differences in the ages of the samples. The measurement of the absorption of essential oils in ultra-violet light as a method for detecting adulterants has been used by K. Dijkstra,[25] who examined some 30 different oils. L. H. Briggs [26] finds that the essential oil of *Phyllocladus alpinus* in concentrated sulphuric acid gives a yellow solution, which turns dark brown on the addition of acetic anhydride and develops a green fluorescence.

J. Suchodolski [55] has described a method for the detection of *ethyl o-phthalate* in alcoholic solutions. After evaporation of the solution to dryness with 1 c.c. of sodium hydroxide, 5 c.c. of concentrated sulphuric acid are added and the mixture is heated to 100° C. for 5 to 10 minutes, 25 to 30 mgrms. of resorcinol being then added and the mixture heated for 5 minutes at 160 to 170° C. A permanent fluorescence appears on the addition of 100 c.c. of water and 50 c.c. of sodium hydroxide solution (*d.* 1·4) if ethyl *o*-phthalate is present. Essential oils and other perfumery compounds give only a transient fluorescence or none at all (see also Radley [152] and Brooks and Bacon [154]).

Hydrocarbons, Mineral Oils and Waxes.—A very strong blue fluorescence is shown by *paraffin hydrocarbons and mineral oils* such as petroleum and machine oils. *Vaseline* has a yellow fluorescence, although when spread out in thin layers it fluoresces blue very strongly. *Ceresin* glows with a strong white light, and yellow ceresin with a dull yellow-brown colour having a purple tinge. The method of examining the oil in a thin layer, by placing a drop on a filter-paper, is useful in this case, for in this way the fluorescence is often greatly increased and admixture or contamination with mineral or edible oils is often detectable; only very small amounts of mineral oil are usually necessary to modify the fluorescence of the edible oil (see also Foods, and p. 190). Similar methods are used for the detection of anthracene in benzene, and a sensitiveness of $1 : 10^7$ or more is attainable.

The fluorescence of various hydrocarbons has been also

examined by E. Galle and others,[27] who found differences between paraffin, ceresin, carnauba, montan wax, beeswax, petroleum spirit and mineral oil (see below). Hexane, pentylene benzene and low-boiling fractions of Pennsylvania oil did not fluoresce. K. W. Haussor [28] investigated the relationship between the fluorescence of solutions of some diphenyl polyenes and the concentration, type of solvent, temperature and state of aggregation; C. Dhéré and A. Raffy [29] have examined hydrocarbons of the rubene family; K. Haberl's observations [30] on the fluorescence of cyclohexane are criticised by R. Padmanabhan [137] who, using a continuous distillation apparatus, found that cyclohexane has no fluorescence, that reported by Haberl being due to photo-chemical decomposition in the region of 2400 to 2600 Å. The fluorescence of octohydro-fluorocyclene in various solvents has been reported by S. Pieńkowski [140] and is practically independent of the solvent used, or of the wave-length of the exciting light.[141]

The dependence of the fluorescence on the degree of purity of the substance concerned is very marked in the case of hydrocarbons. Thus, E. Canals and P. Peyrot [217] found that 9 pure hydrocarbons were non-fluorescent. The examination of oil stains on textiles and the control of oils used in the textile industry are discussed on page 369.

Waxes examined by one of us (J. A. R.) [72] usually have characteristic fluorescence colours, and in some cases the admixture of one with another is detectable. Carnauba wax shows a very bright yellow colour mottled with a faint brown, and in solution, a brilliant milky-blue or bluish-violet is seen. So strong is this fluorescence that the presence of less than 1 per cent. of this wax in admixture with others can be detected in a chloroform solution examined under the lamp. Chinese wax has a white fluorescence with a blue tinge; japan wax, ivory; foreign beeswax, orange-brown; English beeswax, brownish-yellow with a green tinge; white beeswax, white with a blue tinge. Examination of the fluorescence of a chloroform solution enables 10 per cent. of paraffin wax, or less than 1 per cent. of carnauba wax, to be detected in any of the above. The chloroform solutions of most other waxes are practically devoid of fluorescence. E. J. Kraus [124] considers the lamp to be of doubtful value for the characterisation of beeswax, an opinion with which we are in agreement; addition of paraffin or

ceresin to the beeswax may intensify the fluorescence, but the method is uncertain. As pointed out above the detection of small amounts of paraffin wax or carnauba wax in admixture is only possible with solutions, since the examination of films of the waxes and other methods give very poor results. Soaps have been examined by O. Hagen,[125] but his paper deals more with the use of the lamp for accelerated rancidity tests, and gives very little information on the actual fluorescence.

Amino Compounds and Animal Substances.—The fluorescence of amino acids in solution, the intensity of the fluorescence and the absorption of light necessary to produce this fluorescence have been investigated by C. Wiegand,[56] who considers that it originates in the aldehydes formed on irradiation. The results collected for proteins and amino acids by C. Dhéré [145] show that they all fluoresce in shades of blue, green and violet, and the changes in shade which occur on irradiation are shown to be an indication of the corresponding changes undergone by the compounds concerned; the fluorescence of certain proteins and amino acids has also been described by F. Vlès.[143] So long ago as 1911, A. Harden and D. Norris [146] noted that a red colour, having a green fluorescence, is produced when a dilute solution of a protein is shaken with a small amount of 10 per cent. sodium hydroxide and 1 drop of 1 per cent. diacetyl solution (Voges-Proskauer reaction).

Amines, etc.—If fused with dichlorofluoran and zinc chloride, primary and secondary aliphatic amines, respectively, produce a yellow-green or red fluorescence soluble in ethyl alcohol or in hydrochloric acid, whilst aromatic amines produce no fluorescence.[156] H. Eichler [157] found that in the presence of a diazo compound and an excess of alkali the red fluorescence of a slightly acid solution of resorufin (cf. p. 293) disappears. R. Cantieni [159] found that a brown fluorescence is produced by addition of 0·1 to 1 per cent. of *pyridine* to a solution of fructose. If 1 per cent. is exceeded, a gas is evolved and a yellow colour appears, the depth of which is a measure of the pyridine content. N. Prileshaeva [144] has investigated the "anti-Stokes effect" of the fluorescence of aniline vapours, and has found that it reaches a maximum at 250° C. and disappears at 350 to 400° C.

Creatinine shows a blue fluorescence under the lamp, but if a solution in butyric acid is heated at 165 to 170° C. for

10 minutes and the crystalline mass which separates on cooling is recrystallised from alcohol and washed with ether, a distinct yellow-green fluorescence is obtained.[145] The mass has the same composition as creatinine and the colour of the fluorescence is dependent to some extent on the *p*H value of the solution, being unchanged in alkaline solution and bluish in acid. Only certain fatty acids (butyric acid in particular) produce this colour-change, no effect being noticed with inorganic, tartaric or lactic acids (*cf.* p. 294). G. Reif [57] suggests that the colour-change is probably due to a conversion to the enolic form, and that the simultaneous occurrence of creatinine and fatty acids in many foodstuffs may influence the fluorescence of these foodstuffs. Other amino acids, amides and organic bases do not give this reaction. Certain amides, however, have a characteristic fluorescence (Ley and Fisher [87]). T. H. Nunan and J. K. Marsh [126] have examined the vapours of aromatic amines and find that their results confirm previous findings, *viz.*, that substances which fluoresce in solution do so also in the vapour state. The oxidation products formed when indole, and the 3.5- and 7-methyl indoles are irradiated as drops on filter paper have a strong yellow fluorescence, and according to H. Freytag [155] skatole has a bluish fluorescence.

Carvacrol and *malic acid* (see also Foods, p. 179) when heated with concentrated sulphuric acid give yellow resinous compounds and, according to S. Šchlivić and S. Lebedev,[129] the alkaline, dilute aqueous solutions are colourless and have an intense blue fluorescence. The various dicarboxylic acids may also be distinguished by reactions which produce fluorescent end-products.[165]

A number of *animal compounds* have been examined by H. Stübel,[58] including keratin, tyrosine, asparagine, glutin, chitin and elastin, all of which give a light blue fluorescence similar to that shown by plant vitellin. Chitin, when colourless, fluoresces strongly, but the intensity of the fluorescence gradually decreases with increase in colour. Thus E. Merker [59] added increasing amounts of a non-fluorescent dye to a colourless sample of chitin, and obtained a corresponding decrease in fluorescence. *Indoxyl* has a yellow-green fluorescence [145] which changes to a purer green shade if acid and furfuraldehyde are added, and the solution is extracted with carbon disulphide. When heated with

hydrochloric acid, *cholic acid* produces a substance having a green fluorescence.

Albumin fluoresces with a bluish colour and this fluorescence has been utilised to study the age of eggs (see p. 163). L. Mallet [60] has made the interesting observation that when certain hypochlorites in solution are added to albumin, rays of ultra-violet light are emitted, and can be detected by the addition of some fluorescent substance.

Cholesterol (see also Foods, p. 147) has been examined by H. Marcelet [127] who found that pure cholesterol from various sources had a uniform mauve fluorescence, but that impure specimens had a yellow fluorescence and that certain crystals appeared yellow and others mauve. Recrystallisation restores the mauve fluorescence of the impure samples. Several samples of pure cholesterol had a yellow fluorescence and lower melting-points after a year, showing that they had deteriorated. It is interesting to note that cholesterol from the spinal cords (m. pt. of 145·5° C.) behaves exceptionally in that it has a strong blue fluorescence when fresh. It has also been found [171] that after irradiating cholesterol with ultra-violet light or with sunlight, it emits an invisible radiation.

Ergosterol and *Carbohydrates* are dealt with elsewhere (see Foods, Paper), but it may be mentioned here that B. L. Vanzetti [128] found that by treating carbohydrates with concentrated sulphuric acid, diluting and allowing to stand for a few days a blue fluorescence is observed, which decreases and finally disappears after several months. Boiling or sunlight causes it to disappear within an hour. Distinct bands were observed between 5790 and 3900 Å. with maxima at 4500 and 4550 Å. The fluorescent body is the result of partial oxidation of the carbohydrate.

Volumetric Analysis and Fluorescent Organic Indicators.

As already mentioned (p. 289), the fluorescence of a number of substances changes in intensity and/or colour with a change in the *p*H value of the solution, and this phenomenon naturally led to the investigation of the suitability of such substances as fluorescent indicators.[155, 215] It is clearly useless to attempt the titration of liquids such as coloured wines, fruit juices, and dark coloured vinegars with ordinary indicators without diluting the

solution considerably, but after the addition of a suitable fluorescent indicator the titration may be carried out in ultra-violet light with ease in many cases.[89, 132] Other advantages are the small quantity of indicator usually required, and the fact that unlike many indicators of volumetric end-points, the substance used in many cases, does not participate in the reaction itself, but acts indirectly. In this way, two errors usually inherent in the use of indicators are eliminated. It is also possible often to work with very weak solutions of the titrating reagent.

The technique is very simple. A thin-walled conical flask is suitable for the titration-vessel, and it should be supported opposite the beam of ultra-violet light so that the radiations strike the liquid being titrated at an angle of about 30° (*cf.* J. Grant [199]). Quartz is unnecessary if the flask has thin walls and is made of non-fluorescent glass. It is desirable to carry out the titration in the dark, but the device described on page 83 enables the burette to be read under these conditions, or alternatively, the graduation marks on the burette may be rendered visible by smearing them with vaseline, which is highly fluorescent.

In 1926 R. Mellet and A. Bischoff [61] suggested the use of *quinine* for this purpose, while in the same year *umbelliferone* was advocated by R. Robl,[62] and *β-methyl umbelliferone* was suggested by C. Bülow and W. Dieck.[63] The technique is indicated above (see also p. 83).

L. Colombier [64] has investigated the colour-change of the fluorescence with the *p*H value of solutions of *eosin*, *erythrosin*, *fluorescein*, *acridine*, *umbelliferone* and *β-naphthol*, and is of the opinion that none are suitable for the determination of *p*H values, although subsequent workers (see Grant [65]) are not in complete agreement.[206, 214] The best procedure is to " calibrate " the indicators first in solutions containing buffers of known *p*H values. The last three indicators can certainly be used, however, for acidimetric titrations, and umbelliferone and its derivatives, in particular, are excellent for strong acids and bases down to concentrations of 0·001 *N*, and also for 0·01 *N* acetic acid and ammonia. The only reaction in which acridine can be employed with advantage is the titration of ammonia (*e.g.*, 0·002 *N*). Umbelliferone is preferable to β-naphthol for *p*H values over 7, and this indicator is also useful for the titration of drug-house effluents in dilutions of 0·1 *N* to 0·01 *N*.

Table 22 is compiled from the data of the various worke on the subject.[64, 69, 73-86, 131, 158, 163]

TABLE 22.

Indicator.	Colour Change.	pH Range.
Acid-R-phosphine[191] . .	—	6·0 — 7·0
Acridine	Green to violet	4·9 — 5·1
Acridine orange[131, 202] . .	Orange to yellow-green	8·4 — 10·4
Benzoflavin	Yellow to green	0·3 — 1·7
Brilliant diazol yellow[202] .	Colourless to violet	6·5 — 7·5
Chromotropic acid[201] . .	Colourless to blue	3·5 — 4·5
Cleves acid[203] . . .	Colourless to green	6·5 — 7·5
Cotarnine	Yellow to white	12·0 — 13·0
Coumaric acid	Colourless to green	7·2 — 9·0
Coumarin	Weak green to bright green	9·8 — 12·0
Dichlorofluorescein . .	Colourless to green	4·0 — 6·0
3.6-Dihydroxy phthalic dinitrile	Blue to green	5·8 — 8·2
3.6-Dihydroxy phthalimide .	(1) Blue to green (2) Green to yellow-green	Below 2·4 6·0 — 8·0
3.6-Dihydroxy xanthone .	Colourless to blue-violet	5·4 — 7·6
Eosin	Colourless to green	2·5 — 4·5
Eosin and Xylen Cyanol FF in ethyl and n-butyl alcohols[193] . .	Colourless to green	4·0 — 5·0
Eosin yellow (eosin G)[206] .	Colourless to yellow	0 — 3·0
Eosin BN[206]	Colourless to yellow	10·5 — 14·0
Erythrosin	Colourless to green	4·0 — 4·5
4-Ethoxyacridone . .	Green to blue	1·2 — 3·2
Ethoxyphenyl-naphthostilbazonium chloride .	Green to colourless	9 — 11
Fluorescein[209]	Colourless to green	4·0 — 4·5
G-salt[203]	Dull to bright blue	9·0 — 9·5
Magnesium-8-hydroxy quinoline complex[200] .	Colourless to golden	6·5 — 7·5
β-Methyl aesculetin . .	Colourless to blue	4·0 — 6·2
β-Methyl umbelliferone[210, 219]	Colourless to blue	6·5 — 7·5
Naphthazol derivatives[198] .	Colourless to yellow or green	8·2 — 10·0
α-Naphthionic acid[195] . .	Azure blue to green	9 — 11
β-Naphthionic acid . .	Azure blue to violet	12 — 13
β-Naphthol	Colourless to blue	6 — 8
α-Naphthol sulphonic acid .	Dark blue to bright violet	8·0 — 9·0
β-Naphthol sulphonic acid .	Dark blue to bright violet	9·0 — 10·0
1-Naphthol-2-sulphonic acid (Sodium salt) . . .	Dark blue to bright violet	9·0 — 10·0
1-Naphthol-4-sulphonic acid[211]	Colourless to blue	6·0 — 6·5

TABLE 22—*Continued.*

Indicator.	Colour Change.	pH Range.
α-Naphthylamine	Colourless to blue	3·4 — 4·8
β-Naphthylamine	Colourless to violet	2·8 — 4·4
Neville-Winther acid [192]	Colourless to blue	6·0 — 6·5
Orcinaurine [208]	Colourless to green	6·5 — 8·0
Papaverine, oxidised by $KMnO_4$ [207]	Yellow to blue	9·5 — 11·0
Patent phosphine [191]	—	6·0 — 7·0
Phloxine	Colourless to bright yellow	3·4 — 5·0
Quinine [194, 211] (see below)	(1) Blue to violet	5·9 — 6·1
	(2) Violet to colourless	9·5 — 10·0
Quininic acid [205]	Yellow to blue	4·0 — 5·0
R-salt [203]	Dull to bright blue	9·0 — 9·5
Resorufin	Yellow to orange (weak)	4·4 — 6·4
Salicylic acid	Colourless to dark blue	2·5 — 3·5
Schæffer's salt [195]	Violet to green to blue	5·0 — 11·0
SS-acid (sodium salt) [203]	Violet to yellow	10·0 — 12·0
3.6 - Tetramethyldiaminoxan - throne	Green to blue	1·2 — 3·4
Thioflavin [191]	—	6·5 — 7·0
Umbelliferone [194]	Colourless to blue	6·5 — 7·6

With umbelliferone as a fluorescent indicator 1·0 c.c. of an acid solution in 250 c.c. of water may be titrated with 0·01 *N* alkali, and the results obtained are slightly lower than with phenolphthalein, which changes at pH 9 to 10 (see p. 311).

In 1929 J. Eisenbrand [66] showed that *quinine*, which is a diacid base, gives two end-points, one at pH 5·8 to 6·1 (blue to violet, for strong acids) and another at pH 9·5 to 10 (violet to colourless, for weak acids). R. Mellet and Bischoff had previously used quinine and its salts as indicators, and had showed that the nature of the fluorescence changes sharply over certain ranges of concentration of acid, but that it depends mainly on the specific action of the acid concerned, rather than on pH value. Titrations of hydrochloric, sulphuric and oxalic acid with sodium hydroxide solution were carried out successfully, but only poor results were obtained with ammonia. As quinine gives two end-points it has been used in the titration of the first two stages of ionisation of phosphoric acid, and it is particularly useful in turbid solutions.

This change in the fluorescence of quinine suggests the possibility of being able to titrate quinine itself, and Danckwortt [8]

mentioned that investigations on these lines were being carried out in his laboratory. However, one of us (J. Grant [67]) has shown that if quinine is dissolved in a known amount of 0·01 *N* sulphuric acid, the excess of acid can be titrated with 0·01 *N* sodium hydroxide solution in ultra-violet light, with an accuracy of 0·1 c.c. The great advantages of the method are that it may be used in turbid or coloured solutions, and it is unaffected by atmospheric carbon dioxide and the usual errors inherent in ordinary indicators (see Grant [65] and pp. 124 and 167). Where the solution to be titrated is particularly coloured (*e.g.*, with alkaline liquors from the digestion of esparto) J. Grant has found it advantageous to add indicator-powder, *e.g.*, quinine to the liquid in a conical flask. The solid particles are then carried into the foam, and the change in fluorescence of this is more easily observed. The method is of course applicable to all coloured liquids, a trace of saponin being added if these do not foam naturally. Canals and Peyrot [99] found that the intensity of the fluorescence of quinine in dilute sulphuric acid at 18° C. and *p*H 2 is directly proportional to the concentration and may be used for its determination. It may be recalled here (see p. 202, Inorganic) that the fluorescence of inorganic compounds, *e.g.*, uranium salts, is often inhibited by the presence of organic compounds such as quinine and other alkaloids (see below).

Further work by J. Eisenbrand [68] on fluorescent substances as *external indicators* also uses the appearance or disappearance of fluorescence to show the end-point.

A. G. Nasini and P. de Cori,[70] reviewed this work, and made determinations on black liquor, sulphuretted oils, wines, beers, acid solutions of ferrous sulphate and commercial indigo-carmine, and found that the method could be employed with advantage in many of these titrations. In addition they also mention the use of coumarin, naphthol sulphonic acid, and dichloro-fluorescein.

Y. Volmar [71] also recommends the general use of quinine and umbelliferone for various titrations, but considers that uranyl salts are poorer as indicators owing to the influence of other salts on the fluorescence. He therefore proposes to use this method for the detection of such ions (see also Inorganic Chemistry, p. 202). The inactivating ions are Cl', Br', CN', S'', SCN' and $Fe(CN)_6''''$, whilst the activating ions are ClO'_3, BrO'_3,

IO'_3, NO'_3, SO''_4, PO'''_4. It is found that the amount of the former necessary for the suppression of the fluorescence of a given quantity of uranyl sulphate is inversely proportional to the chemical equivalent of the ions. The organic compounds containing halogens such as chloroform, bromoform, chloral, etc., are without effect, and hence the presence of mineral halogens may be detected in admixture with organic halogen derivatives. So far as other organic compounds (*e.g.*, phenols and hydrocarbons) are concerned, Eisenbrand [148] has been unable to trace any relationship between structure and inhibiting power, except that hydrocarbons with high molecular weights or with unsaturated conjugated double-bands appear to be most effective.

Fink and Hoerburger have shown that if the variation of fluorescence is measured and plotted against the corresponding variations in *p*H value of the solution, curves are obtained which are often characteristic for the substance concerned, and they propose this as a method of identification. In particular R. Kuhn and G. Moruzzi [130] noted fluorescence minima in certain cases where there was an iso-electric region, and like Eisenbrand (p. 153) they used the method to determine ionisation-constants, notably of the flavins. Analogous methods have been used successfully in connection with the change in absorption spectrum and *p*H value of ordinary indicators (Grant [65]).

As already indicated, the use of fluorescent indicators is of value in the *titration of coloured wines* (see also p. 174). Titration of red wines with phenolphthalein sometimes gives high values, but with umbelliferone Y. Volmar and J. M. Clavera [73, 74] obtained results in close agreement with the values given by potentiometric titration. Thus, 0·5 c.c. is shaken in a vacuum, to remove carbon dioxide, and 2 to 5 drops of an alcoholic solution of 1 part in 1,000,000 of umbelliferone are added, the wine being then titrated with 0·1 *N* or 0·05 *N* sodium hydroxide solution under the lamp until a blue fluorescence just appears. Acridine gives correct results only if compared with a solution adjusted to the end-point. These indicators are, however, useless for the determination of the acidity of beer, as beer is itself fluorescent, but they may be used in the titration of Catalonian, Estraburgian, Alsatian and other commercial red wines. Acridine (1 : 500 in alcohol) gives the poorest results, but although umbelliferone give results 0·5 to 1 c.c. less than phenolphthalein

(7 wines), they are only 0·1 c.c. below the end-point corresponding with *p*H 7 as determined potentiometrically.

J. M. Gallart [75] criticised these results, but he does not contest the use of fluorescence methods in wine titration so much as the nature of the true end-point of wine acidity, and therefore there is no need to discuss his work here. In any case, his criticisms are answered by Clavera,[76] who indicates the agreement of his own work with that of other workers (Semichon and Flanzy, Colombier,[64] etc.); the umbelliferone method may therefore be confidently recommended.

Adsorption Indicators.[158]—It is now well known that many precipitation reactions may be carried out with the aid of internal indicators, which show the end-point as the result of a change in colour arising from differences in the degree of adsorption of the indicator on the precipitate formed. As a number of the indicators suggested for use when these titrations are made in ordinary light happen to be fluorescent in filtered ultra-violet light, it is not surprising that attempts have been made to use them in these radiations, especially where coloured or turbid solutions are concerned.

Some of the best examples of the applications of these methods are in connection with the determination of halogen compounds [213]; in many instances these have the advantage that they may be carried out in the presence of nitric acid, which renders the usual potassium chromate indicator useless. Thus, C. F. M. Rose,[196] V. Collier,[197] and H. R. Fleck, R. F. G. Holness and A. M. Ward [200] have all used (*e.g.*, a 0·005 per cent. solution of) dichloro-fluorescein for the direct titration of chlorides (*e.g.*, in body fluids). The change is from a yellow-green fluorescence to non-fluorescence when 0·025 *N* silver nitrate solution is added rapidly to the chloride solution. The value is in good agreement with that obtained by visual methods. Eosin (0·1 per cent.) changes from a golden fluorescence to colourless in the titration of 0·025 *N* bromides or iodides, but it is important to note that as the actual end-point depends on the rate at which the silver nitrate is added, the solutions should be standardised using the same technique as is employed for the test itself. The best results are obtained if the silver nitrate solution is added rapidly.

J. Grant [199] has also obtained good results with quinine sulphate, the change in fluorescence being from pale blue to dull

purple when a 0·1 N solution of sodium chloride is run into silver nitrate in the presence of a pinch of the solid salt. As the change is visible in the presence of many dyestuffs (*e.g.*, 0·2 per cent. solutions of caramel, nigrosine, tartrazine, etc.) it may be used for the determination of chlorides in dyestuffs, especially where these have been " diluted " with salt. An end-point is also obtainable for the titration of zinc sulphate by a ferrocyanide, the blue fluorescence of the quinine sulphate disappearing when the ferrocyanide ions are present in an excess. A sharp end-point results in cold solution, and the result, though low, is consistently low. It is doubtful whether quinine sulphate behaves as a true adsorption indicator; the principal factor appears to be the modifications in its fluorescence which occur in the presence of an excess of certain ions. S. N. Roy [204] records that fluorescein or eosin in the presence of $Bi^{\cdots}$ ions serves as an indicator for the titration of nitric acid with sodium hydroxide or sodium carbonate.

Orthotungstates may be titrated with lead nitrate, with quinine or umbelliferone as a fluorescent indicator,[194] and the morin reagent (p. 205) may be used to indicate the end-point in the titration of aluminium salts with a neutral 0·1 N solution of sodium fluoride in the presence of potassium chloride. In the latter case the green fluorescence persists until all the aluminium has been precipitated as Na_3AlF_6; this method may also be operated colorimetrically (Okac [216]). The sodium salt of fluorescein has been used as a fluorescent indicator in the titration of lead salts with sodium carbonate (*cf.* S. N. Roy [218]).

Further work on fluorescence indicators for the titration of amino-compounds and their salts, and of carbonates, is due to Y. Volmar and M. Widder,[78-80] Volmar, [81-84] J. Volmar,[77] J. Eisenbrand,[85, 86] and J. Muir [88] quotes a private communication from the laboratory of Messrs. Boots Pure Drug Co., Ltd., where the fluorescence of quinine is used to determine the correct *p*H value of the end-point of an indicator. Mixed fluorescent indicators have also been used [212] as an aid to the determination both of *p*H values and of volumetric end-points; examples are fluorescein with methyl red or methyl orange. β-Methyl umbelliferone has been used to show the end-point in the determination of the saponification value of paprika oils.[219]

It is appropriate to mention here J. Eisenbrand's work on

fluorescence as an aid to the *measurement of ionisation-constants*. Eisenbrand considers that the contradictory nature of the data at present available is due to certain disturbing factors such as light absorption, which his own apparatus enabled him to eliminate or control. The fluorescence intensity of a solution of quinine sulphate was found to increase almost linearly with increase in concentration, and is also affected by the absorption of light by the solution according to Beer's Law (*i.e.*, the intensity of the transmitted light is inversely proportional to the thickness of the absorbing layer and to the concentration of the absorbing solution). Eisenbrand determined the ionisation constants of quinine sulphate, methoxyquinoline and β-naphthol, from the formula $K = \frac{(0{\cdot}01\, I\, (\mathrm{OH}))}{(1 - 0{\cdot}01\, I)}$, where K is the ionisation-constant, (OH) the hydroxyl-ion concentration, and I the intensity of the fluorescence, and his results are in good agreement with those obtained by other methods. He considers that, contrary to opinions expressed in some quarters, a relation does exist between the intensity of fluorescence and ion formation in solution (see also p. 289). In their work on the flavins R. Kuhn and G. Moruzzi[153] found that, within certain limits of pH value, the intensity of fluorescence is exactly proportional to the concentration of pigment. The decreases in intensity on the acid and alkaline sides may be used to determine the number of electrically-neutral molecules, and thence the ionisation-constant.

REFERENCES.

[1] R. Robl, *Zeit. angew. Chem.*, 1926, **39**, 608.

[2] H. Kauffmann and A. Beisswenger, *Zeit. phys. Chem.*, 1904, **50**, 350.

[3] L. Pincussen, "Biologische Lichtwirkungen, Ihre Physikalischen und Chemischen Grundlagen" (Munich and Wiesbaden: J. F. Bergmann, 1920).

[4] E. Bayle and R. Fabre, *Jour. Pharm. et Chim.*, 1925, **1**, 248.

[5] S. Dutt, *Jour. Indian Chem. Soc.*, 1930, **7**, 505 (*Brit. Chem. Abst. A.*, 1930, 1345).

[6] E. Bayle and R. Fabre, *Compt. rend. Acad. Sci.*, 1924, **178**, 632.

[7] G. T. Dragone, *Atti R. Accad. Lincei*, 1928, **8**, 602.

[8] P. W. Danckwortt, " Lumineszenz-Analyse im Filtrierten Ultravioletten Licht " (*Akademische Verlagsgesellschaft*, Leipzig, 1934).

[9] L. Ekhert, *Pharm. Zentralhalle*, 1931, **72**, 51.

[10] F. Kirchhoff, *Kautschuk*, 1928, **4**, 24.

[11] J. Eisenbrand, *Pharm. Ztg.*, 1930, **75**, 1033 (*Brit. Chem. Abst. A.*, 1930, 1259).

[12] H. Ley and R. Dreinhöfer, *Zeit. wiss. Phot.*, 1930, **29**, 134.

[13] F. G. Segitz, " Ueber Chromoisomere Alkali-Enolate des Benzoylacetons " (Inaug.-Diss., Leipzig, 1928).
[14] H. O. Albrecht, *Zeit. phys. Chem.*, 1928, **136**, 321.
[15] A. Schontag and H. Fischer, *ibid.*, 1938, **39B**, 411.
[16] M. Déribéré, *T.I.B.A.*, 1936, **40**, 557.
[17] F. Schütz, *Papier-Fabr.*, 1938, **36**, 55.
[18] N. Prileshaeva and R. Tschubarov, *Acta Physicochim. U.S.S.R.*, 1935, **1**, 777.
[19] E. Canals, P. Peyrot and R. Noël, *Compt. rend.*, 1935, **201**, 208, 1488.
[20] P. Karrer and H. Fritzsche, *Helv. Chim. Acta*, 1936, **19**, 481.
[21] H. von Euler, K. M. Brandt and G. Neumüller, *Biochem. Zeit.*, 1935, **281**, 206.
[22] W. Czapska-Oarkienwicz, *Bull. int. acad. polon. Classe sci. math. nat.*, 1935, **A**, 445.
[23] J. Weiss, *Trans. Faraday Soc.*, 1936, **32**, 1331.
[24] K. S. Gururaza Das, *Proc. Indian Acad. Sci.*, 1937, **6**, 24.
[25] K. Dijkstra, *Pharm. Weekblad*, 1936, **73**, 502.
[26] L. H. Briggs, *J. Soc. Chem. Ind.*, 1937, **56**, 137T.
[27] E. Galle, R. Klath and W. Friedl, *Montan Rundschau*, 1935, **27**, 14.
[28] K. W. Hausser, *Zeit. phys. Chem.*, 1935, **29B**, 417.
[29] C. Dhéré and A. Raffy, *Bull. Soc. Chim.*, 1935, **2**, 1424.
[30] K. Haberl, *Ann. Physik*, 1934, **21**, 301.
[31] L. Meunier and A. Bonnet, *Compt. rend.*, 1925, **180**, 2038 ; **181**, 465 ; *Jour. Intl. Soc. Leather Trades Chemists*, 1925, **9**, 340.
[32] F. T. Addyman, *Dyer and Calico Printer*, 1929, **61**, 266.
[33] G. T. Dragone, *Atti R. Accad. Lincei*, 1927, **6**, 179.
[34] L. Petri, *ibid.*, 1927, **5**, 136.
[35] L. Petri, *Boll. R. Staz. Industria Pelli.*, 1927, **5**, 201.
[36] K. Noack, *Biochem. Zeit.*, 1927, **183**, 135.
[37] A. Wilschke, *Zeit. wiss. Mikrosk.*, 1914, **31**, 338.
[38] M. Tswett, *Ber. Deut. botan. Ges.*, 1911, **29**, 744.
[39] C. Dhéré and M. Fontaine, *Ann. Inst. Océanographique*, 1931, **10**, 9.
[40] C. Dhéré and M. Fontaine, *Compt. rend.*, 1931, **192**, 1131.
[41] C. Dhéré, *ibid.*, 1931, **192**, 1496.
[42] C. Dhéré, *ibid.*, 1914, **158**, 64 ; Abderhalden's " Handbuch der Biologischen Arbeitsmethoden," II, **3**, (iv), (Berlin : Urban and Schwarzenberg, 1933).
[43] C. Dhéré and W. Rogowski, *Compt. rend.*, 1912, **155**, 653.
[44] C. Dhéré and L. Ryncki, *ibid.*, 1913, **157**, 501.
[45] K. Stern, *Zeit. f. Botan.*, 1921, **13**, 193.
[46] J. Wlodek, *Bull. Ac. Pol. Sc. B.*, 1924, 407.
[47] A. Müller, *Die Riechstoffindustrie*, 1927, **2**, 139.
[48] R. Escourrou, *Chim. et Ind.*, 1930, **24**, 779.
[49] R. Mellet and A. Bischoff, *Pharm. Acta Helv.*, 1929, **4**, 134.
[50] E. Ekman and A. Samyschlayewa, *Die Riechstoffindustrie*, 1930, **5**, 164.
[51] E. Ekman and A. Samyschlayewa, *ibid.*, 1930, **5**, 182.
[52] E. Ekman and A. Samyschlayewa, *ibid.*, 1930, **5**, 196 ; **6**, 140 ; 1933, **8**, 221.
[53] C. P. Wimmer and M. H. Kennedy, *Perf. Ess. Oil Record*, 1930, **21**, 163.
[54] T. Pavolini, *Ind. Ol. mineral. e grass.*, 1930, **10**, 41.
[55] J. Suchodolski, *Przemysl. Chem.*, 1930, **14**, 265.
[56] C. Wiegand, *Pflüger's Arch. f. Physiol.*, 1929, **231**, 378.
[57] G. Reif, *Zeit. Unters. Lebensm.*, 1929, **58**, 28.

[58] H. Stübel, *Pflüger's Arch. f. Physiol.*, 1911, 142, 1.
[59] E. Merker, *Zoöl. Anzeiger.*, 1929, 4, 181.
[60] L. Mallet, *Compt. rend.*, 1927, 185, 352.
[61] R. Mellet and A. Bischoff, *ibid.*, 1926, 182, 1616.
[62] R. Robl, *Ber.*, 1926, 59, (B), 1725.
[63] C. Bülow and W. Dieck, *Zeit. anal. Chem.*, 1928, 75, 81.
[64] L. Colombier, *Ann. Falsif.*, 1931, 24, 89.
[65] J. Grant, " The Measurement of Hydrogen Ion Concentration " (London: Longmans, Green & Co., 1930).
[66] J. Eisenbrand, *Pharm. Ztg.*, 1929, 74, 249.
[67] J. Grant, *Analyst*, 1931, 56, 653.
[68] J. Eisenbrand, *Zeit. angew. Chem.*, 1929, 42, 447.
[69] G. A. Bravo, *Ind. Chimica*, 1929, 4, 8.
[70] A. G. Nasini and P. de Cori, *Atti* III *Congresso Naz. Chim. pur. applicata*, 1929, 698; 1930, 668.
[71] Y. Volmar, *Arch. Phys. Biol.*, 1928, 6, 61.
[72] J. A. Radley, *Analyst*, 1932, 57, 626.
[73] Y. Volmar and J. M. Clavera, *Jour. Pharm. Chim.*, 1931, 13, 561.
[74] Y. Volmar and J. M. Clavera, *Anales Fisica Y Química*, 1931, 29, 247 (*Brit. Chem. Abst. B.*, 1931, 693).
[75] J. M. Gallart, *ibid.*, 1931, 29, 490 (*Brit. Chem. Abst., B.*, 1931, 1117).
[76] J. M. Clavera, *Anales Fisica Y Química*, 1931, 29, 494.
[77] J. Volmar, *Jour. Pharm. Chim.*, 1927, 5, 435.
[78] Y. Volmar and M. Widder, *Bull. Soc. Chim.*, 1929, 45, 696.
[79] Y. Volmar and M. Widder, *ibid.*, 1929, 45, 130.
[80] Y. Volmar and M. Widder, *ibid.*, 1928, 43, 813.
[81] Y. Volmar, *ibid.*, 1927, 41, 774.
[82] Y. Volmar, *Chimie Industrielle*, 1928, 21, 160.
[83] Y. Volmar, *Bull. Soc. Chim. France*, 1927, 41, 302, 774.
[84] Y. Volmar, *ibid.*, 1927, 42, 1155.
[85] J. Eisenbrand, *Pharm. Ztg.*, 1930, 75, 1033.
[86] J. Eisenbrand, *Zeit. phys. Chem.*, 1929, 144, 441.
[87] H. Ley and W. Fisher, *Ber.*, 1913, 46, 327, 3627.
[88] J. Muir, *Ind. Chem.*, 1933, 9, 49.
[89] W. Holthof, *Z. anal. Chem.*, 1933, 91, 263.
[90] P. Müller, *Ann. Chim. Anal.*, 1933, 15, 11.
[91] J. Chloupek, *Chem. Listy*, 1933, 27, 493.
[92] H. Eichler, *Zeit. anal. Chem.*, 1934, 96, 17, 22 and 98.
[93] P. W. Danckwortt, *ibid.*, 1934, 97, 185.
[94] T. Tokio and M. Ishii, *Jour. Pharm. Soc. Japan*, 1929, 49, 475.
[95] G. de Haas, *Pharm. Weekblad*, 1934, 68, 29.
[96] E. Tommila, *Suomen Kem.*, 1934, 7, 85B. (*Brit. Chem. Abst. A.*, 1934, 758).
[97] E. Eegriue, *Zeit. anal. Chem.*, 1933, 95, 323.
[98] H. G. Crone and R. G. W. Norrish, *Nature*, 1933, 132, 241; 1934, 133, 833.
[99] E. Canals and P. Peyrot, *Compt. rend.*, 1934, 198, 746.
[100] R. Ţiţeica, *Bul. Soc. române Fiz.*, 1933, 35, 149.
[101] R. Tomaschek, " Phosphoreszenz, Fluoreszenz, und Chemische Reaktionsleuchten; Handbuch d. phys. Optik.," Vol. 2 (Leipzig, 1927).
[102] F. Henrich and F. Braun, *Jour. prakt. Chem.*, 1934, 139, 338.
[103] A. J. Allen, R. Franklin and E. McDonald, *Jour. Franklin. Inst.*, 1933, 215, 705.

[104] A. and L. Lumière and A. Seyewetz, *Bull. Soc. Chim.*, 1925, **37**, 700.
[105] L. J. Heidt and G. S. Forbes, *Jour. Amer. Chem. Soc.*, 1933, **55**, 2701.
[106] P. Swings, "Exposés de Physique Moléculaire" (Paris: Hermann et Cie, 1933).
[107] E. Canals and P. Peyrot, *Compt. rend.*, 1934, **198**, 1992.
[108] J. Moir, *Trans. Roy. Soc. S. Africa*, 1924, **12**, 45.
[109] R. Kuhn and F. Bär, *Ber.*, 1934, **67B**, 898.
[110] J. A. Radley, *Chem. and Druggist*, 1933, **129**, 340.
[111] Zickgraf, *Arch. Pharm.*, 1931, **269**, 427.
[112] U. Drehmann, *ibid.*, 1930, **268**, 585.
[113] C. Dhéré, *Compt. rend.*, 1934, **198**, 126.
[114] S. Hilpert, H. Hofmeier and A. Wolter, *Ber.*, 1931, **64**, 2570.
[115] H. Kautsky and A. Hirsch, *Naturwiss.*, 1931, **19**, 40.
[116] H. Kautsky, and A. Hirsch, *Ber.*, 1931, **64B**, 2677; *Biochem. Z.*, 1935, **277**, 250.
[117] A. Stoll, *Naturwiss.*, 1932, **20**, 955.
[118] A. Dusseau, *Compt. rend.*, 1930, **190**, 68.
[119] H. V. Knorr and V. M. Albers, *Phys. Rev.*, 1933, **43**, 379.
[120] F. E. Lloyd, *Science*, 1924, **59**, 241.
[121] E. Vigliani, *Minerva Med.*, 1933, **2**, 674.
[122] K. G. Stern and E. R. Holiday, *Ber.*, 1934, **67B**, 1104.
[123] Wimmer and H. Goodman, *Amer. Jour. Phys. Therapy*, 1928, **5**, 262.
[124] E. J. Kraus, *Fettchem. Umschau*, 1933, **40**, 50.
[125] O. Hagen, *Seifensieder-Ztg.*, 1931, **37** 38, 38.
[126] T. H. Nunan and J. K. Marsh, *Jour. Chem. Soc.*, 1924, **125**, 2123.
[127] H. Marcelet, *Bull. Soc. Chim. Biol.*, 1933, **15**, 379.
[128] B. L. Vanzetti, *Rend. semin. fac. sci. univ. Cagliari*, 1933, **3**, 47.
[129] S. Šchlivić and S. Lebedev, *Bull. Soc. Chim. Yougoslav.*, 1933, **4**, 133.
[130] R. Ţiţeica, *Compt. rend.*, 1934, **199**, 458.
[131] K. A. Jensen, *Zeit. Anal. Chem.*, 1933, **94**, 177.
[132] S. Malowan, *Chem. Ztg.*, 1933, **57**, 76.
[133] E. Herzog, *Rév. Gén. Mat. Col.*, 1933, **37**, 824.
[134] J. Bouchard, *Compt. rend.*, 1934, **198**, 649; **199**, 43, 460.
[135] F. Perrin, *ibid.*, 1924, **178**, 1928.
[136] F. G. H. Macrae, *Amer. Dyestuff Reporter*, 1933, **22**, 647.
[137] R. Padmanabhan, *Proc. Indian Acad. Sci.*, 1935, **2A**, 209.
[138] S. M. Mitra, *Indian Jour. Phys.*, 1933, **8**, 171; 1934, **8**, 445.
[139] W. König and W. Regner, *Ber.*, 1930, **63B**, 2823.
[140] S. Pienkowski, *Bull. int. acad. polon. sci. Classe sci. math. nat.*, 1937, **A**, 269.
[141] M. Makoviecka, *Acta phys. polon.*, 1934, **2**, 357.
[142] H. Kautsky, A. Hirsch and W. Baumeister, *Ber.*, 1931, **64**, 2053.
[143] F. Vlès, *Compt. rend.*, 1936, **202**, 2184.
[144] N. Prileshaeva, *Acta Physicochim. U.S.S.R.*, 1935, **1**, 785.
[145] C. Dhéré, Abderhalden's "Handbuch der Biologischen Arbeitsmethoden," II, **3**, 4, (Berlin: Urban and Schwarzenberg, 1933).
[146] A. Harden and D. Norris, *Jour. Physiol.*, 1911, **42**, 322.
[147] F. Feigl, V. Anger and O. Frehden, *Mikrochem.*, 1934-35, **17**, 29.
[148] J. Eisenbrand, *Zeit. physikal. Chem.*, 1933, **22B**, 145.
[149] N. D. Costeanu and A. S. Cocosinschi, *Bul. Fac. Stünte Cernauti*, 1931, **5**, 169 (through *Brit. Chem. Abst. A.*, 1934, 346).
[150] Schimmel and Co., Leipzig, Ann. Rept., 1928 (trans. by R. E. Kremers).
[151] R. A. Morton, *Perf. and Ess. Oil Record*, 1929, Aug.

[152] J. A. Radley, *Soap Trade and Perf. Rev.*, 1933 ; *Mnfg. Chem.*, 1934, 4, 296.
[153] R. Kuhn and G. Moruzzi, *Ber.*, 1934, **67B**, 888.
[154] B. T. Brooks and R. F. Bacon, *Jour. Ind. Eng. Chem.*, 1914, **6**, 623.
[155] H. Freytag, *Zeit. Anal. Chem.*, 1935, **103**, 334.
[156] F. Feigl, V. Anger and R. Zappert, *Mikrochem.*, 1934, **16**, 67.
[157] H. Eichler, *Zeit. Anal. Chem.*, 1934, **99**, 27, 348.
[158] H. R. Fleck, R. F. G. Holness and A. M. Ward, *Analyst*, 1935, **60**, 32.
[159] R. Cantieni, *Helv. Chem. Acta*, 1934, **17**, 1492.
[160] E. Eegriwe, *Zeit. Anal. Chem.*, 1935, **100**, 31.
[161] K. Gleu and W. Petsch, *Zeit. angew. Chem.*, 1935, **48**, 57.
[162] A. Cotton and others, "La Polarimétrie en Lumière Ultra-Violette" (Paris, 1934).
[163] A. G. Pukirev and M. S. Maslova, *Zavod. Lab.*, 1934, **3**, 1038.
[164] E. Bottini, *Ann. della Sperim. Agrar.*, 1934, **15**, 61 ; H. Nicol, *Perf. and Ess. Oil Record*, 1935, **26**, 85.
[165] F. Feigl, V. Anger and O. Frehden, *Mikrochem.*, 1935, **17**, 29.
[166] J. Voss, *Angew Bot.*, 1934, **16**, 510.
[167] H. Eichler, *Zeit. Anal. chem.*, 1935, **103**, 425.
[168] Salm, *Zeit. Phys. Chem.*, 1907, **57**, 500.
[169] H. Eichler, *Chem. Ztg.*, 1936, **60**, 357.
[170] K. Kalle, *Naturwiss.*, 1937, **25**, 61.
[171] A. H. Roffo and A. E. Roffo, *Compt. rend.*, 1936, **201**, 1301.
[172] G. Brooks, *Compt. rend.*, 1938, **207**, 693.
[173] H. Kautsky and R. Hormuth, *Naturwiss.*, 1936, **24**, 650.
[174] H. Kautsky and R. Hormuth, *Biochem. Zeit.*, 1937, **291**, 285.
[175] H. Kautsky and W. Flesch., *ibid.*, 1936, **284**, 412.
[176] J. Franck and H. Levi, *Naturwiss.*, 1935, **23**, 226, 229.
[177] J. Franck and R. W. Wood, *J. Chem. Phys.*, 1936, **4**, 551.
[178] J. Franck, *Naturwiss.*, 1935, **23**, 389.
[179] H. Kautsky, *ibid.*, 1935, **23**, 389.
[180] H. Kautsky and A. Hirsch, *Biochem. Zeit.*, 1935, **277**, 250, 373.
[181] J. Weiss, *Nature*, 1936, **138**, 80.
[182] K. Weber, *ibid.*, 1936, **137**, 870.
[183] O. Richter, *Sitz. Akad. Wiss. Wien. Mathnaturwiss.*, 1935, **144**, 157.
[184] H. V. Knorr and V. M. Albers, *Phys. Rev.*, 1933, (ii), **43**, 379.
[185] H. V. Knorr, V. M. Albers and P. Rothemund, *ibid.*, 1935, (ii), **47**, 198.
[186] C. Dhéré and A. Raffy, *Bull. Soc. Chim. biol.*, 1935, **17**, 1385.
[187] H. Kautsky and A. Marx, *Biochem. Zeit.*, 1937, **290**, 248.
[188] H. V. Knorr and V. M. Albers, *Phys. Rev.*, 1936, (ii), **49**, 420.
[189] J. Franck and K. F. Herzfeld, *J. Phys. Chem.*, 1937, **41**, 97.
[190] J. Franck and K. F. Herzfeld, *J. Chem. Phys.*, 1937, **5**, 237.
[191] A. Borgialli, *Boll. Uff. Staz. Sperim. Ind. Pelli*, 1935, **13**, 245.
[192] M. Déribéré, *Ann. Chim. Anal.*, 1936, **18**, 117.
[193] T. M. B. Marshall, *J. Soc. Dyers and Col.*, 1936, **52**, 299.
[194] A. del Campo and F. Sierra, *Anal. Fís. Quím.*, 1935, **33**, 364.
[195] M. Déribéré, *Ann. Chim. Anal.*, 1936, **18**, 173.
[196] C. F. M. Rose, *Biochem. Jour.*, 1936, **30**, 1140.
[197] V. Collier, *Jour. Biol. Chem.*, 1936, **115**, 239.
[198] M. Déribéré, *Ann. Chim. Anal.*, 1936, **18**, 37.
[199] J. Grant, *Analyst*, 1937, **62**, 285.
[200] H. R. Fleck, R. F. G. Holness and A. M. Ward, *ibid.*, 1935, **60**, 32.
[201] E. A. Kocsis and Z. von S. Nagy, *Zeit. Anal. Chem.*, 1937, **108**, 317.

[202] M. Déribéré, *Ann. Chim. Anal.*, 1937, **19**, 290.

[203] M. Déribéré, *ibid.*, p. 262.

[204] S. N. Roy, *Jour. Ind. Chem. Soc.*, 1938, **15**, 165.

[205] L. Szebellédy and J. Jonás, *Zeit. Anal. Chem.*, 1938, **113**, 266.

[206] M. Déribéré, *Bull. Assoc. Chim.*, 1938, **55**, 275 ; *Document Sci.*, 1937, **7**, 241.

[207] L. Szebellédy and J. Jónás, *Zeit. Anal. Chem.*, 1938, **113**, 326.

[208] J. Jónás and L. Szebellédy, *ibid.*, p. 422.

[209] F. Krüger, *Ber.*, 1876, **9**, 1572.

[210] A. G. Pukirev and M. S. Maslova, *Zavodsk. Lab.*, 1934, **3**, 1038.

[211] M. Déribéré, *Ann. Chim. Anal.*, 1936, **18**, 120.

[212] R. Burstein, *Zavodsk. Lab.*, 1937, **6**, 825 ; *Acta Phys. Chim. U.R.S.S.*, 1937, **7**, 311.

[213] H. Gotō, *J. Chem. Soc. Japan*, 1938, **59**, 365, 797.

[214] H. Linser, *Biochem. Zeit.*, 1932, **244**, 157.

[215] W. Mecklenburg and S. Valentiner, *Phys. Zeit.*, 1914, **15**, 267.

[216] Okac, *Coll. Czechoslovak Chem. Comm.*, 1938, **10**, 177.

[217] E. Canals and P. Peyrot, *Compt. rend.*, 1936, **203**, 998.

[218] S. N. Roy, *Jour. Ind. Chem. Soc.*, 1936, **13**, 40.

[219] E. A. Kocsis and P. Vass, *Zeit. Unters. Lebensm.*, 1936, **71**, 442.

[220] E. J. Bowen, *Nature*, 1938, **142**, 1081.

[221] M. Haitinger, *Mikrochem.*, 1934-35, **16**, 321.

ADDITIONAL REFERENCES.

J. D. B. H. van Assenraad, *Pharm. Tijdschr. Nederl.-Ind.*, 1931, **8**, 207, 260 (Acetone and acetic acid).

N. Schoorl, *Pharm. Weekblad*, 1931, 68, 279 (Fluorescence of β-naphthol).

M. François and L. Séguin, *Bull. Soc. chim. France*, 1931, **49**, 680 (Phenol).

S. J. Wawilow, *Compt. rend. U.S.S.R.*, 1937, **16**, 255 (Polarised fluorescence of dyestuffs).

P. G. Glakhow and J. A. Khvostikow, *ibid.*, p. 259 (Polarised fluorescence of dyestuffs).

H. Chromse and W. Lutzenberger, *Zeit. anorg. Chem.*, 1938, **238**, 236 (Activation of inorganic substances by organic phosphors).

J. A. Radley, *Chem. Age*, 15 2, 36 (Ultra-violet light as an aid in volumetric work).

Y. Volmar, *Document Sci.*, 1936, **5**, 33 ; *Chim. et Ind.*, 1936, **37**, 446 (Acid-alkali fluorescence indicators).

A. Jermstadt, *Dansk. Tidskr. Farmaz.*, 1932, **6**, 141 (Fluorescence indicators).

J. Grant, *Current Sci.*, 1936, **4**, 801 (Fluorescence analysis as an aid to chemical analysis).

P. Ramart-Lucas, *Compt. rend.*, 1937, **205**, 1409 (The quinonoid form of fluorescein is fluorescent, and the lactoid form is non-fluorescent).

G. Denigès, *Bull. trav. soc. pharm. Bordeaux*, 1938, **76**, 65 (Quinine and halide ions).

S. Cryglewicz, *Acta Phys. Polon.*, 1937, **6**, 210 (Anthracene).

L. Szebellédy and K. Sik, *Magyar Gyóg. Társaság Értesitoze*, 1938, **14**, 387, 391, 405, 409, 413, 418 (Fluorescent indicators).

CHAPTER XV.

PAINTS AND VARNISHES.

THE variety of substances employed in the colour and varnish industry offers a wide scope for the use of the ultra-violet lamp, particularly for the examination of raw materials. Oils, waxes, resins, pigments and drying agents can all be examined in this manner, and valuable information is obtained as to the source and, in many cases, the nature of the industrial treatment.[14] According to one opinion [30] the lamp can give very useful results for the examination of raw materials used in the paint and varnish industry, although it is of less use with processed or finished products. The application of ultra-violet light to the testing of artist's paints is described by K. Wurth,[31] and also on page 283. Fading and ageing tests are dealt with by J. Grant,[28, 53] and on pages 344 and 394. V. G. Jolly [16, 17] has investigated the use of the lamp for artificial weathering and accelerated-ageing tests on paints (*cf.* Rubber, p. 353 and Paper, p. 344), and considers the lamp more uniform than the sun in its action. The carbon arc is considered the best source of light by this worker, as it is more like the sun in its spectral characteristics than the mercury arc (Fig. 10, p. 28), and the latter also favours "chalking." The paints were tested under the lamp in conjunction with "artificial rain," but the method needs strict standardisation, and in any case can only be used for qualitative and comparative work. In the case of lacquers nitrocellulose alone changes in strength, viscosity, stability and alcohol-solubility on exposure to ultra-violet light, but these changes are inhibited by plasticisers and especially by tricresyl phosphate.[26, 27] On the other hand P. Snitter [18] considers the lamp poor for ageing tests, and J. Grant [53] (p. 345) deals with alternative methods involving fluorescence analysis which, although evolved in connection with the study of paper, have also been applied to inks, and could probably be extended to certain paints and varnishes.

Solvents.—K. Schmidinger[1] has examined 185 various solvents, resins, waxes and colours used in the industry, and has tabulated the natural colour against the colour of the fluorescence in ultra-violet light. The solvents, as a whole, show various shades of blue, sometimes with a green tinge and often with a somewhat milky appearance. Table 23 shows the results for those of the fifty-five solvents examined by Schmidinger which show a fairly strong fluorescence.

TABLE 23.

Pure benzene.	Fairly strong violet.
Motor benzol (B.-Pt. 80-160° C.).	Weak sky-blue.
Lac benzene (B.-Pt. 140-190° C.).	Rather strong, milky violet-blue.
Butanol.	Fairly strong blue.
Benzyl alcohol.	Rather strong violet.
Chlorotoluene.	Fairly strong, milky-grey with a bluish tinge.
Cyclohexanone.	Rather strong milky violet-blue.
Dichlorohydrin (tech.).	Brownish-grey.
Epichlorohydrin (tech.).	Fairly strong violet-blue.
Fenchyl acetate.	Fairly strong, milky bluish-green.
Pure glycerol (S.G. 1·26).	Rather strong violet-blue.
Methyl hexalin.	Fairly strong greenish-blue.
Methyl hexanone.	Fairly strong milky sky-blue.
Solvent naphtha II.	Fairly strong milky sky-blue.
Pentachloroethylene.	Fairly strong milky greenish-blue.
Pure pyridine.	Weak milky-yellow.
Tetralin.	Rather strong milky sky-blue.
Turpentine (American).	Fairly strong violet-blue.
Turpentine (German).	Weak greenish-blue.

Resins.—Schmidinger also examined resins in the massive form. He records the appearance of the freshly-broken face of the mass of resin, as in many cases this differs from that of a surface which has been exposed to the air for some length of time. Resins from Brazil, Sierra Leone and Benguella all appear in their natural colours under the lamp, but the broken faces show various shades of blue. Dammar *AC* and *E* both have a bright blue fluorescence, whilst Sumatra Dammar that has been melted appears grey-yellow. Raw Elemi shows a milky-white fluorescence with a bluish tinge, and Siam stick lac, seed lac, knot lac and ruby shellac all fluoresce in shades of gold colour.

Of the *synthetic resins* Bakelite-*A* appears very dark olive, whilst Albertol-III *L* and *K6S* appear dull grey-blue and dark olive-brown, respectively. Freshly-broken surfaces of Albertol-III *L*

show up much brighter than old surfaces, and those of Albertol *K6S* have a milky grey-blue colour. Other synthetic resins are discussed under amber (*vide infra*).

H. Wolff and W. Toeldte [2] have also examined resins in the form of powder, in solution in butyl acetate, and finally, as a film left by the evaporation of the butyl acetate. It is, however, always preferable to examine the powdered resin, since it has a much stronger fluorescence than the lump form ; the surface of the solutions was also found to be convenient for observations. It was noted that where there is a rather strong fluorescence the colour is generally a shade of blue, often with a greenish or reddish tinge. By far the strongest fluorescence is given by Albertol, with ester resin and Congo copal next in order of intensity.

It was also observed that the colour of the fluorescence of a resin is sometimes changed when the resin is dissolved ; for instance, powdered colophony shows a bluish fluorescence which changes to a green shade in solution. The residue after evaporation of solutions of colophony fluoresced as shown in Table 23A :—

TABLE 23A.

Colophony.	Bluish-green.
Half-neutralised.	Greener, and a little brighter.
Almost neutral.	Still more green, but dull.
Excess alkali.	Still more green and duller.

The orange-red fluorescence of lac changes to a green fluorescence on titration with alkali, the change being fairly sharp and taking place at a slightly lower *p*H value than when thymol blue is used as an external indicator. An end-point between these two values is obtained if a small amount of β-naphthol is added to the solution, and N. N. Murty and H. K. Sen [43] find that the results agree very well with those obtained with the quinhydrone electrode, even with very dark lacs.

By the combination of capillary and fluorescence analysis (see pp. 58 and 59), H. Wolff [3] has obtained some useful data relating to resins. No clearly-defined zones were obtained, but preponderating quantities of one resin in a mixture were apparent from the appearance of the fluorescence-colour, and useful information was provided in this manner. E. Stock [32] has examined " Almerioina Gutta " a Belgian Congo resin, and has

tabulated its fluorescence properties. The use of different solvents to obtain various fluorescent effects with the same resin has been applied by this worker to the examination of the resin from *Symphonia globulifera*.

A. Tschirch and E. Stock [25] have noted the *triboluminescence of resins*, *i.e.*, the light given out when they are rubbed in a mortar in a dark room. They record the following fluorescences of various resins :—

Weak yellow : Benzoin (Siam), crude amber, colophony (French, Portuguese, Spanish, German, Grecian), shellac.
Green : Benzoin (Sumatra), aloe (Barbados), ammoniacum, colophony (America), pure pimaric and abietic acids, copal (Zanzibar and Congo), mastic, sandarac, stick-lac.
Grey : aloe (Cape).
Violet : guaiacum.
White : hard brown copal (Kauri), natural olibanum, shellac (Reinharz).
Little or none : Asafoetida, aloe (Zanzibar), fused amber, dragon's blood, elemi, galbanum, galipot, euphorbium, natural myrrh, jalap, copal (Manila, Brazil), and tolu balsam.

These workers consider that the origin of the resins may often be determined in this way, *e.g.*, with colophony, shellac, copals, benzoin and aloes. They also used 10 per cent. alcoholic solutions for the examination of the resins by the capillary strip method (see p. 58). Resins and plastics have been examined by M. Déribéré,[33] and F. Krämer [34] has used the resorcinol method for the detection of resins made from phthalic anhydride and has obtained marked differences in fluorescence. The test is carried out by heating the resin with a few crystals of resorcinol and a drop of concentrated sulphuric acid (see p. 294).

Amber (see also p. 272).—Stokes noted many years ago that amber is fluorescent, and G. C. Williamson,[13] who has made a special study of this substance, quotes a report on his specimens by the National Physical Laboratory, Teddington, as follows : " All of these fluoresce with a bluish-grey light, the intensity of fluorescence ranging from that produced by ordinary lubricating oil at the maximum to that emitted by vaseline at the minimum." Williamson then says : " Such a long series of experiments has, to the best of my knowledge, never hitherto been carried out, and

in fact the fluorescence of amber has been overlooked by the majority of writers on the subject."

The order of (intensity of) fluorescence, according to this most interesting and valuable report from Teddington, runs as follows :—

"(1) Yellow rumanite; (2) osseous succinite; (3) mottled rumanite. There is very little difference between 1 and 3."

It is also considered that a weak chemi-luminescence is emitted by amber owing to oxidation.

The use of ultra-violet radiation to distinguish between *natural and artificial amber* has been investigated by G. Kostka.[4] Natural amber fluoresces with a yellowish-green to bluish-white colour, and pressed amber, or ambroid, behaves similarly, although the fluorescence is less pronounced. Clouded amber has a somewhat different fluorescence from that of the clear variety, but the difference is probably mainly one of degree only, and is due to structural differences rather than to the presence of impurities.

Phenol-formaldehyde condensation products absorb the rays, but no fluorescence is excited. *Urea-formaldehyde condensation products*, on the other hand, emit a slight fluorescence which is easily distinguished from that shown by genuine amber. This difference provides a rapid means of distinction between urea- and phenol-formaldehyde condensation products. Resin-like materials prepared from natural products such as casein and cellulose derivatives give a bluish-white or blue fluorescence. H. Wiesenthal[5] has also worked on the fluorescence and other characteristics of artificial substances used in the place of amber. Another resin having no fluorescence is the recently introduced Perspex, which is a polymerised polyvinyl compound, and which shows excellent transparency to ultra-violet light.

Driers.—Schmidinger (see also H. Wolff and W. Toeldte[6]) gives the following fluorescent colours of a number of siccatives :—

Cobalt resinate (precipitated).	Dark blue.
Zinc resinate (precipitated).	Ivory. Broken face, white with bluish tinge.
Manganese resinate (precipitated).	Dark violet.
Lead resinate (precipitated).	Dark ivory.
Lead and manganese resinates (mixed).	Dark green-grey. Broken face, milky-white.
Cobalt linoleate.	Brownish-olive. Broken face, violet-blue.
Lead linoleate.	Olive. Broken face, violet-grey with brown tinge.
Manganese linoleate.	Dark with green tinge. Broken face, ivory with strong violet tinge.

The colours of the luminescence differ sufficiently to enable the pure substance to be recognised at once under the lamp, whilst the resinates of zinc and lead may easily be recognised in admixture with other drying agents.

Oils.—The fluorescence of a number of raw, refined and mixed oils, extracting agents, wood oils, and pigments have been published by A. Wagner.[7] Linseed oil appears bright milky-yellow and rape oil has a brownish-yellow fluorescence ; refined rape, linseed, arachis and soya bean oils, however, all show a bright blue fluorescence, so that it is impossible to differentiate them. Raw linseed oil adulterated with increasing quantities of arachis oil shows at first a yellowish fluorescence, which is gradually replaced by a blue colour as the percentage of arachis oil is increased. Raw rape oil containing 30 per cent. of linseed, arachis or soya bean oil, shows no distinct modification in colour, all the mixtures appearing brownish-yellow (*cf.* p. 147).

Plasticisers, also, often show characteristic colours, but in this case a warning is necessary since (as W. M. Münzinger[15] has shown) exposure to ultra-violet light may change this colour. Thus, for example, castor oil turns from light blue to grey, and tolyl phosphate from violet to brown. This phenomenon is not unknown in other connections (see p. 350).

Paints and Varnishes.—Paints and varnishes have been examined by H. Wolff and W. Toeldte,[8] who state that little or no differentiation can be obtained, although the results of other investigators on the detection of adulteration of linseed oil are confirmed to some extent.

A clear differentiation between resinous and resin-free varnishes is however, obtained, since the former gives a bluish colour whilst the latter show a dirty greenish fluorescence.

Pigments.—A. Eibner and his co-workers [9] have examined a large number of opaque, transparent, coloured and white pigments from commercial and natural sources. They find that although most of the coloured pigments do not fluoresce, some of the whites show a distinct fluorescence ; for example, heavy spar in the powdered form shows a brownish-violet colour, a similar but less intense colour being also shown by precipitated barium sulphate. Titanium white (extra *XP*) can be differentiated from white lead, a deep violet being obtained in the first case and a bright reddish-brown fluorescence in the second. Zinc white

has a vivid yellowish-green colour, and, according to M. J. Schoen and J. Rinse,[10] the presence of 25 per cent. of this compound in titanium white causes a colour change from deep purple to greenish-yellow.

Some of the results obtained by Schmidinger in his examination of a number of mineral pigments and their substitutes are given in Table 24, but care must be taken in drawing conclusions, as the presence of small amounts of impurities (*e.g.*, in samples from different sources) produces far-reaching effects on the colour of the fluorescence. The statement sometimes made that lithopones which are fast to light are not fluorescent has not been confirmed. The fluorescence of some of the substitutes is so intense that chemical distinguishing tests need not be applied (*cf.* p. 340).

TABLE 24.

(See also Photograph No. 19.)

Pigments.	*Fluorescence Colour.*
Ground Shale.	Dark blue.
Fixed White	Fairly strong violet.
Light spar.	Fairly strong violet.
Precipitated chalk.	Black.
Ground chalk.	Red with brownish tinge.
Natural chalk.	Dark yellow.
Siliceous chalk.	Fairly strong red-violet.
Kaolin.	Deep reddish-violet.
Talcum.	Dirty red-violet.
White lead.	Bright rose-brown with violet tinge.
Aluminium hydroxide.	Light blue.
Titanium white extra (*XP*).	Deep violet-blue.
Titanium dioxide (pure).	Deep dark violet.
Lithopone.	Blue-green with a brown, yellow or red tinge according to source and purity.
Pure cinnabar.	Dark.
Cinnabar substitute (Lithol red).	Cinnabar-red.
,, ,, (Permanent red).	Carmine.
Pure red lead.	Dark.
Red lead substitute (Lac red).	Orange-red.
,, ,, (Helio-orange).	Reddish-brown.
Zinc yellow (zinc chromate, pure).	Dark.
,, ,, 1. Spar 6.	Dark.
,, ,, 1. Spar 15.	Dark greyish-green.
Ultramarine blue, pure.	Dark blue-violet.
,, ,, substitute (Victoria blue).	Dark blue-violet, with reddish tinge.
Zinc sulphide.	Orange.
Magnesium oxide.	Blue-green.
Magnesium carbonate.	Violet.

F. Schmidt [23] considers that by illumination in ultra-violet light useful information concerning the structure of pigment particles 0·1μ in diameter may be obtained, while G. S. Haslam and C. H. Hall [24] have also used the fluorescence microscope (see p. 78) for the determination of particle size. The examination of zinc oxides under the lamp to determine the effect of particle size on the fluorescence has been undertaken by several workers (see pp. 324, 355), but so many factors seem to come into play with this substance that it does not appear safe as yet to draw any definite conclusions.

A large amount of work, both quantitative and qualitative, has been carried out on pigments obtained commercially and prepared in the pure state in the laboratory by E. Beutel and A. Kutzelnigg,[11, 12] the principal object being the determination of the origin of paintings (see p. 283). Zinc whites usually appeared yellow even when present in minute quantities,[12] and lead carbonates had a beige-coloured fluorescence, and could often be identified in mixtures with barium sulphate. The presence of zinc white could be detected with certainty, but the colour of white lead is not so definite, as exposure to air probably induces reactions which may alter the colour of the fluorescence; variations are therefore found as the painting ages. Mixtures of titanium white with zinc oxide show a progressive variation in fluorescence colour when the amount of titanium white is decreased, the colour being deep-violet initially, and finally yellow.

Interesting information on the fluorescence of zinc, cadmium and other pigments is given by L. Levy and D. W. West,[35] who have investigated the use of the fluorescent pigments to rectify the green colour of the light from the mercury arc lamp, and so to bring the light emitted by the complete unit nearer to white light by super-imposing the fluorescent light on that of the lamp. The fluorescence of pigments is also discussed by R. Toussaint.[36]

Interesting experiments have been carried out in various countries on the use of fluorescent and phosphorescent pigments and paints for use in air raid shelters and for military purposes. In France the movement of troops in lorries during a complete blackout was successfully accomplished by fitting filters to the headlamps and marking the streets with fluorescent paints.

The advantage of phosphorescent paints, having an effective afterglow of 5 to 8 hours, in air-raid shelters is self-evident when

the possibility of the interruption of electric services is considered. A number of papers have appeared on the preparation of luminous paints, [29, 37-39] and P. M. Wolf and M. Riehl [38] consider strontium and zinc sulphides to be the most suitable pigments for use in this connection. The preparation of a standard material is, however, a very difficult matter, as great attention must be paid to the purity of materials and to the temperature cycles used in their preparation (see p. 203). Improved effects are claimed by B. Steiger and A. Lorenz [40] by depositing the active material on an aluminium oxide base. L. Vanino [52] has given a brief summary of the qualitative analysis of various pigments which are activated by small amounts of impurities, and the colour and maximum wave-length of the characteristic fluorescence, together with the activator, is tabulated for each pigment. He considers that the lamp allows added dyestuffs to be detected, as well as any radioactive compounds added as excitants.

Various binding agents have been used as a vehicle to carry a fluorescent substance which may be a soluble dyestuff or a pigment. Polystyrene,[41] cellulose acetate,[42, 43] polyvinyl compounds,[44] oil-modified glyptal resins [45, 46] and urea-aldehyde resins [47] have all been protected by patents. One patent [48] claims Rhodamine B–500 (in conjunction with lead chromate) to increase the fluorescent effect of a condensation product of citric acid with a glycol or glycerol. The finished film may be overlayered with another film containing a dyestuff, which may be selected so as to absorb any particular colour desired and to modify the final fluorescence.[49, 50] Brilliant reflex and irridescent fluorescence effects are claimed [57] to be produced by incorporating in a lacquer compounds such as fluorene, phenanthrene, anthracene or carbazole, which crystallise in the film on drying. The I.G. Farbenind.[19] incorporate styrene or vinyl naphthalene, with sulphuric acid (as a catalyst) and polymerise by heating. F. W. V. Fitzgerald [20] adds 2 per cent. of quinine and a small amount of radioactive material to zinc sulphide paints to prolong the duration of luminosity, and sulphides of the alkaline earth metals are also used.

Another patent by the I.G. Farbenind.[21] concerns the manufacture of luminous paints which are also waterproof, and in a paper by A. W. van Henckeroth [22] vehicles for luminous paints are described.

Reference may also be made to Chapter VIII, in particular to page 201, and to the work of G. S. Haslam and C. H. Hall [24] on the use of the fluorescence microscope for the study of the particle size of pigments.

REFERENCES.

1 K. Schmidinger, *Farben-Ztg.*, 1926, **31**, 2451.

2 H. Wolff and W. Toeldte, *ibid.*, 1926, **31**, 2503.

3 H. Wolff, " Die natürlichen Harze." Monographien aus dem Gebiete der Fett-Chemie (Wiss. Verlagsanstalt), Stuttgart, 1928.

4 G. Kostka, *Chem. Ztg.*, 1929, **53**, 117, 138.

5 H. Wiesenthal, *Kunstoffe*, 1929, **19**, 56.

6 H. Wolff and W. Toeldte, *Farben-Ztg.*, 1926, **32**, 80.

7 A. Wagner, *Chem. Ztg.*, 1921, no. 41.

8 H. Wolff and W. Toeldte, *Farbe und Lack*, 1926, 509.

9 A. Eibner, L. Widenmayer and A. Stois, *Farben-Ztg.*, 1926, **31**, 2399.

10 M. J. Schoen and J. Rinse, *Chem. Weekblad*, 1929, **26**, 321.

11 E. Beutel and A. Kutzelnigg, *Monatsh.*, 1931, **57**, 9 ; *ibid.*, 1932, **61**, 69.

12 E. Beutel and A. Kutzelnigg, *Mikrochem.*, 1931, **9**, 360; *Acad. Sci., Vienna*, May, 1932 ; *Z. anorg. Chem.*, 1931, **201**, 323.

13 G. C. Williamson, " The Book of Amber " (London : Benn).

14 P. W. Danckwortt, *Farben-Chem.*, 1932, **3**, 140.

15 W. M. Münzinger, *Chem. Ztg.*, 1932, **56**, 851.

16 V. G. Jolly, *Jour. Soc. Chem. Ind.*, 1933, **52**, 330T.

17 G. L. Clark and H. L. Tstchentke, *Jour. Ind. Eng. Chem.*, 1929, **21**, 621.

18 P. Snitter, *Bull. Inst. Pin.*, 1933, p. 223.

19 I.G. Farbenind., E.P. 400,068, 1/3/1933.

20 F. W. V. Fitzgerald, E.P. 402,777, 27/2/1932.

21 I.G. Farbenind., E.P. 403,233, 16/1/1933.

22 A. W. van Henckeroth, *Amer. Paint and Varn. Manfrs. Assoc., Circular* No. 440, July, 1933, p. 23.

23 F. Schmidt, *Chem. Fab.*, 1933, **6**, 349.

24 G. S. Haslam and C. H. Hall, *Jour. Opt. Soc. Amer.*, 1934, **24**, 14.

25 A. Tschirch and E. Stock, " Die Harze " (Berlin : Borntraeger, 1935).

26 *Manchester Guardian*, Commercial, 10th Feb., 1934.

27 A. Kraus, *Farbe und Lack.*, 1933, pp. 545, 556.

28 J. Grant, *Oil and Colour Trades Jour.*, 1934, **85**, 1601 (Accelerated fading tests on paints and paper).

29 H. Silman, *Paint Manf.*, 1935, **5**, 5.

30 Anon, *Paint Oil. Chem. Rev.*, 1937, **99**, 64.

31 K. Wurth, *Chem. Ztg.*, 1938, **62**, 589.

32 E. Stock, *Farben-Ztg.*, 1937, **42**, 259, 549.

33 M. Déribéré, *Rév. gén. Mat. Plast.*, 1938, **42**, (Feb.) ; *Caout. et Gutta-Percha*, 1937, **34**, 20.

34 F. Krämer, *Farben Ztg.*, 1938, **40**, 1142.

35 L. Levy and D. W. West, *Jour. Soc. Col. Dyers*, 1936, **52**, 262.

36 R. Toussaint, *Peint. Pig. et Vernis*, 1938, **15**, 90.

37 Anon, *Chem. Industries*, 1936, **39**, 371.

38 P. M. Wolf and N. Riehl, *Gas Maske*, 1936, 8, 102.

39 Anon, *Paint Manf.*, 1936, **6**, 355.

[40] B. Steiger and A. Lorenz, F.P. 827,719, 1936.
[41] Philips' Gloeilampenfab., E.P. 452,097, 1936.
[42] Compagnie des Lampes, F.P. 827,586, 1937; Brit. Appl. 27,087/37.
[43] N. N. Murty and H. K. Sen, *Analyst*, 1938, 63, 181.
[44] Jakob Nussbaum, F.P. 809,182, 1937.
[45] Bandolux, E.P. 487,510, 1938.
[46] Canadian Gen. Elec. Co., Can. P. 360,941; *Chem. Zentr.*, 1937, 108 (i), 4300.
[47] Helita Soc. France, F.P. 802,627, 1935.
[48] Philips' Gloeilamp., F.P. 760,224, 1933; F.P. 800,577, 1936; Sw. P. 188,326, 1935.
[49] Philips' Gloeilamp., E.P. 483,662, 1938.
[50] Heyden, Anm. C. 48,522 IVb/22f(10).
[51] Prod. Chim. de Laire, F.P. 812,954, 1936; *S.N.F. Bull. Tech. No.* B.D. 135.
[52] L. Vanino, *Chem. Ztg.*, 1935, 59, 656.
[53] J. Grant, "Books and Documents. Dating, Permanence and Preservation" (London: Grafton & Co.), 2nd Edn., 1939.

ADDITIONAL REFERENCES.

P. W. Danckwortt, *Farb. Chem.*, 1932 3, 140 (through *Chem. Zentr.*, 1932, (i), 3233).
H. Wolff, *Deut. Farb.-Ztg.*, 1931, 67, 89 (Resins).
H. Wagner, "Die Körperfarben," Stuttgart: *Wiss. Verlagsges. m.b.H.*, 1928 (Differentiation of pigments).
E. Beutel and A. Kutzelnigg, *Monatsh.*, 1932, 61, 69 (Zinc oxides).
W. L. Carver, *Auto. Ind.*, 1934, May, No. 19 (Car-body finishes).
A. Kufferath, *Farbe und Lack.*, 1935, 473 (General).
J. Grant, *Text. Colorist*, 1936, 58, 154 (Paints and pigments).
J. Grant, *Paint Manf.*, 1937, 7, 340 (General, including inks).
G. Martin, *Rév. Gén. Mat. Col.*, 1938, 42, 81 (Printing inks and defects).
E. Holzinger, *Chem. Ztg.*, 1932, 56, 1022 (Fluorescence and phosphorescence of zinc oxides).
A. Kutzelnigg, *Monatsh. f. Chem.*, 1934, 64, 61 (Alteration of fluorescence of zinc oxides owing to mechanical treatment).
A. Kutzelnigg, *ibid.*, 1934, 64, 74 (Fluorescence of zinc oxides at the temperature of liquid air).
F. Schmidt, *Chem. Fabr.*, 1933, 6, 351 (Testing Pigments).
R. Roley, *Amer. Ink Maker*, 1936, 16, 18 (Fluorescent inks).
J. Elms, *Rév. Gén. Mat. Plast.*, 1938, 14, 154 (Fluorescence of pigments used in plastics and varnishes).
Anon, *Sci. Sect. Nat. Paint, Var. Assoc., Circ.* 568, 1938, 209; through *Brit. Chem. Abst. B.*, 1939, 76 (Evaluation of covering-power).
Anon, *ibid., Circ.* 546, 1937, 279; *Circ.* 568, 1938, 337; through *Brit. Chem. Abst. B.*, 1938, 190; 1939, 76 (Identification of oils and resins used in paints).

CHAPTER XVI.

PAPER, CELLULOSE AND ALLIED INDUSTRIES.

ONE of us (J. G.[45, 51]) has recently made a close study of the applications of fluorescence analysis to the paper and allied industries, and except where otherwise stated the majority of the observations reported below may be taken as having been put to practical confirmation. The fluorescence of a sheet of finished paper can of necessity convey very little information when judged on its own because it is the resultant of the individual effects of each of the numerous constituents present, and these in turn are dependent on the nature, origin, amount and purity of the constituent and the processing it has undergone. Where, however, a standardised furnish is used (*e.g.*, in newsprint mills), variations in fluorescence have a greater significance, *e.g.*, J. F. Smith has stated [46] that addition of so little as 2 per cent. of sulphite (as " broke ") is detectable. If some generalisation is desired, it may be said that the colour of the fluorescence of the paper depends on the materials and the intensity on the processing, but this is a broad statement which does not always apply, *e.g.*, in the case of coloured papers, where the effect of the small amount of dye used may preponderate over all others.

On the other hand, the method is most valuable for comparative work, for control of the purity and processing of raw materials, and as an instrument of investigation, and it also has indirect uses as a means of carrying out physical tests. In the method of treatment adopted below the materials and stages of manufacture are dealt with in normal sequence.

Raw Materials.—The botanical study of woods used in the paper industry is dealt with in Chapter III, but further investigations were carried out by M. Fallot [9] and by H. Wislicenus.[10] Acacia wood gave an intense yellow fluorescence, industrial bleached fibres a fairly bright violet fluorescence, and raw cellulose a dark brown fluorescence. Forty samples of wood fibres, which

had been carefully purified in the laboratory, fluoresced with a rose colour, and it is therefore apparent that the origin of the sample cannot normally be ascertained from its fluorescence. Wislicenus also considers that woods may be distinguished by the fluorescence of the pith and cambium rings (see also p. 106), and the contribution of wood flavones to the total effect observed has been investigated by F. V. Lutati.[23]

Reference may be made to Chapter XVIII for observations on rags, but little work has been carried out on other materials such as esparto.

Half-Stuffs.—The colours and intensity of the fluorescence of some half-stuffs employed in paper manufacture are summarised in Table 25 (due to Klein [13]) :—

TABLE 25.

Half-stuff.	Description.	Fluorescence.
Kraft.	Bleached.	Dirty white.
Linen.	Unbleached.	Grey to violet.
Linen.	Bleached.	Grey to white.
Cotton.	Unbleached.	Reddish-grey to grey.
Cotton.	Bleached.	Red to grey.
Sulphite cellulose	Unbleached.	Intense violet to reddish-violet.
Sulphite cellulose	Bleached.	Yellow, blue, grey or white.
Alpha cellulose.	Bleached.	Pale, dirty violet.
Straw pulp.	Bleached.	White.
Ground logwood.	Unbleached.	Brown.
Ground pine.	Unbleached.	Mouse-grey to blue-grey.
Esparto.	Bleached.	White to bluish-white.
Manilla.	Unbleached.	Red-brown to grey-brown.

H. Pringsheim and O. Gerngross [32] give results of tests on a number of degradation-products of cellulose and similar substances, and find that in general such degradation is accompanied by an increase in the intensity of the fluorescence. Neither this work nor Table 25, however, brings out fully the remarkable effect of bleaching on the fluorescence of wood pulp, the vivid bluish-white glow being replaced by a dull dirty yellow. The change is so marked and the correspondence with bleachability so close, that it is possible to grade pulps into four categories, ranging from strong, through easy-bleaching, to bleached and super-bleached pulps (see Photograph No. 11, facing p. 400). Many pulp-

mills are now turning out so uniform a product that the fluorescence of sheets drawn from a delivery may be used as a routine check on bleachability and an actual bleaching test is thus avoided. Kimreuther, Schulze and Nippe [25] have also endeavoured to trace a relationship between the nature of the fluorescence and the chlorine number. There appears to be some connection between the degradation produced by drastic cooking and by bleaching, since both processes appear to destroy the fluorescent material. The latter is, however, far more effective, and so little as 0·5 per cent. of chlorine will remove the blue glow almost completely from a strong pulp.

It is interesting to note that there is a decrease in the intensity of the fluorescence after heating at 105° C. for an hour, or at 155° C. for half an hour. A diminution of the intensity also takes place after exposure of the sample to sunlight or to the rays from the ultra-violet lamp; the former is distinctly perceptible after 4 to 8 hours, and the latter after 15 minutes or less, and 20 hours of exposure to sunlight, it is stated, repress the fluorescence of sulphite cellulose completely. These facts should be borne in mind when exposing pulps for the above tests. This point is also emphasised by H. G. Klein [18] and by V. Hottenroth.[24]

Fluorescence microscopy (see p. 78), however, probably offers the most promising field for the investigation of fibres of all kinds, and some conspicuous successes have already been achieved, notably by F. Noss and H. Sadler,[26] and by B. Schulze and E. Göthel.[27, 29] The usual technique is employed, the primary fluorescence being used to distinguish bleached from unbleached pulps as indicated above; incidentally, the shade and brightness of the fluorescence at this stage are related to the length and degree of the cook. A dyestuff is then added in order to differentiate the various kinds of bleached pulp (secondary fluorescence). Of the twenty-two dyes tested Rhodamine-6GD (extra) in a 0·05 per cent. solution was found to give the best results (see Table 26) after a staining period of 2 minutes.

Schulze and Göthel confirmed these results as a whole, and were even able to apply the primary fluorescence tests quantitatively, but difficulties arose in certain cases, notably the quantitative differentiation of bleached sulphite and sulphate pulps from soft woods, especially in the presence of unbleached sulphite. In cases where rhodamine is used, however, one of us (J. G. [28]) has

found that different results may be obtained with dyes of different origins; in general the above results were confirmed. A practical application of the same method is due to B. Schulze,[57] who summarises comparisons between this and other microscope methods, and the chemical values (*e.g.*, lignin-contents and bleachability values) of sulphate and sulphite pulps of various bleachabilities. In this case, both the primary fluorescence and the secondary fluorescence obtained after staining with 0·05 and 0·01 per cent. Rhodamine-6GD "extra," were taken into account.

A selection of the results obtained with a large number of dyes on twenty-two paper fibres are summarised in Table 26.

TABLE 26.

Fibre.	Primary Fluorescence.	Secondary Fluorescence.
Sulphite (U.B.) .	Deep blue-violet.	(1) Brown-yellow. (2) Wine-red (orange and yellow with soft pulps). (3) Wine-red to violet.
Sulphite (B.) .	Yellow to bright green-blue.	(1), (2) and (3) Yellow-green.
Sulphate (U.B.) .	Straw-yellow.	(1) Brown-yellow. (2) Wine-red (intensity depends on strength). (3) Wine-red to violet.
Sulphate (B.) .	Bright blue-white.	(1) Brown-yellow. (2) Wine-red. (3) Green-yellow.
α-Cellulose pulp (B.) . .	Pale dirty violet.	(1) Wine-red.
Straw . . .	Yellow.	(1) Yellow-brown.
Jute . . .	White to blue.	(1) Golden-brown.
Gampi and Mitsumata . .	Blue-white.	(1) and (2) Golden-yellow.
Kodzu . .	Blue-white.	(1) Green-yellow.
Linters. . .	Less intense and greyer than cotton (*infra*).	(1) Dirty brown. (2) If followed by alkali, blue-violet.[47]
Linen . .	Blue-white.	(1) Green-yellow. (2) If followed by alkali, red.[47]
Ramie. . .	Yellow to green.	(1) Green-yellow.

Note.—(1) represents Flavophosphine-4G; (2) Rhodamine-6GD (extra); and (3) Sulphorhodamine-G.

Beech, chestnut, eucalyptus, esparto, bamboo, sugar cellulose, maize, peat and cotton all have a blue-white primary fluorescence

and a yellow-brown secondary fluorescence with Flavophosphine-4G. A hot 1 per cent. solution of Flavophosphine-4G (conc.) gave similar results to the rhodamine, and basic dyes were, in general, found to be preferable to acid or direct dyes. Interesting differentiations between the internal structures of the fibres (*e.g.*, the parenchyma and fibrillæ) were also obtainable in some cases, and Brilliant Dianil Green-G could be used to distinguish summer-wood (blue) from spring-wood (yellow) independently of the cooking conditions; andansonia (light blue) from manilla and jute (blue-green); and white from brown mechanical pulp (blue and yellow-green, respectively); on the other hand, Geranin-G gave a colour with unbleached sulphate pulps which varied from pink to green according to the degree of delignification. Oxy-dianil yellow-O has been used in this way, and oxin sulphate is an aid to the distinction between cotton and linen.[53] Similar methods have been applied to Textiles (p. 360).

H. G. Klein [13] finds that the fluorescence is affected by the chemical treatment of the samples (*e.g.*, with acid or alkali), by heating, or by exposure to light. The alteration due to bleaching is very marked, and slight but distinct variations were noted in the fluorescence of a number of samples after they had been treated with 1 per cent. solutions of hydrochloric acid, nitric acid, sodium hydroxide or ammonia. The moisture content was found to have no appreciable effect (*vide supra*), but changes occurred on heating the samples at 55° C., 105° C., and 155° C. Thus 1 hour at 55° C. produced a brightening of the fluorescence in one series of half-stuffs, but no marked variation in another, although after treatment at 105° C. and 155° C., the fluorescence colours of all the samples were somewhat duller and some showed a change in colour tone.

K. Berndt [14] and also Kimreuther and his co-workers [25] (see p. 337) attempted to determine the *degree of cooking of cellulose pulp* by means of the lamp, and also found that the intensity of the fluorescence of unbleached sulphite pulp is an indication of the relative degree of cooking. Fresh pulp should be used, as exposure to air and ultra-violet light induces oxidation and, as mentioned above, this also produces an alteration in the fluorescence. Attempts to reproduce these results (by J. Grant) have not been particularly successful, and Gerngross,[15] Rassow and Brandau,[16] and Klein,[17, 18] have all found that the intensity

depends also on the degree to which the pulp is cooked, and hence that reliable results are obtained only under strictly comparable conditions. Rosin or natural resins will also modify the normal fluorescence of a pulp, and the present writer has found that the fluorescence of that portion of the natural pulp resins which is soluble in alcohol is a much duller yellow than the ether-soluble portion, which is usually held responsible for "pitch" troubles.

Sulphite cellulose liquor [59] is discussed under Tans (p. 217), and the titration of coloured digester or soda-recovery liquors is described on p. 314 (see Grant [45]).

The optical studies of H. Ress [30] on *beating* also include ultra-violet light, and in all cases increased beating was found to produce a more uniform fluorescence, *i.e.*, a disappearance of the "marbled" effect of unbeaten pulp; the colour-intensity also increases and brightens, although, of course, on prolonged exposure it falls off again.

Loadings present quite a promising field of investigation as may be gathered from the sections on paint pigments and inorganic chemistry. One of us (J. G.) has examined a large number (see Photograph No. 19, facing p. 400), and was able to distinguish good china clay (weak violet) from mineral white (bright violet), talc (weak blue-grey), blanc-fixe (strong blue-violet), and chalk (brighter blue). Chalk, zinc sulphide and zinc oxide were found to vary in appearance according to their degrees of purity, but the grades of titanium oxides could not be distinguished either from one another or from china clay. The method is very useful for checking deliveries, and in one instance in the writer's experience [31] it was possible to detect tinting dyes added to china clay. In such cases if experimental sheets containing the genuine and adulterated material are compared in ultra-violet light the differences are very striking, and the differences are also sometimes visible in the ash obtained after incineration.

Colouring Matters.[54]—The identification of dyes is discussed on p. 387, but Photographs Nos. 6 and 10 (facing p. 400) indicate the value of the method for this purpose, as well as for checking deliveries. In such cases it is an advantage if possible to compare dyed and undyed sheets of paper, in the former case both for the questioned dye and a standard. A similar procedure is applied to pigments, and the writer found that a sample of ultramarine

which had been brought to the correct shade by addition of a minute quantity of a dye, appeared pale red under the lamp instead of blue-violet.

Examination of Finished Papers.[33, 34]—In 1893 W. N. Hartley[1] noted that unsized paper has a bright fluorescence, but the fluorescometry of cellulose and its derivatives has been more carefully studied by S. Judd Lewis,[3-8] who has elaborated an accurate method for the examination of *papers and fabrics* and has set this branch of the subject on a quantitative basis. The source of the ultra-violet rays used by this worker was a rotating tungsten arc which gives a very intense spectrum between 2000 and 3300 Å., a range which, it will be noted, is not that generally employed in fluorescence analysis. Fig. 21 will make clear the arrange-

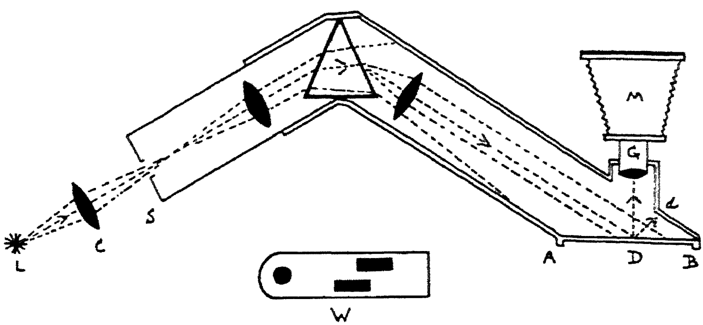


FIG. 21.

ment of the apparatus used in these experiments. The ultra-violet rays from L pass through the lens C and the aperture S to the quartz prism, and the resulting spectrum falls at an angle on a sheet of the paper or fabric (D), the resulting fluorescence being photographed by means of a camera (M) placed at right angles to the sample. The lens of the camera is made of ordinary glass, so that the small amount of ultra-violet light reflected from the sample is absorbed and does not affect the photographic plate, which registers only the light due to the fluorescence of the paper. In this way a photograph is obtained which shows that only waves of a certain wave-length produce fluorescence, the intensity of this fluorescence being shown by the density of the negative. These intensities are then compared with those from a standard paper which is practically

non-fluorescent, and are then plotted against the wave-length of the exciting light. This is accomplished by means of the aperture-wedge (W), which fits into the slit and enables either or both of the papers to be exposed.

A number of curves for various papers and fabrics were obtained, and it was found that various classes of cellulose derivatives gave characteristic curves, and, what is very important, that these depend on the nature of the treatment in the mill, *e.g.*, parchmentisation, etc. Air-dry specimens were used, as it was found that the fluorescence is not affected appreciably by the moisture in the air. A number of samples were examined in the form of fabric, powder and film, but it was found that the uniformity of the results was not affected by the physical state of the material.

Blemishes on paper are frequently difficult to identify without tedious microscopic examination, but the writer (J. G.) has used ultra-violet light very successfully here, notably to distinguish oil spots (which preserve their bright blue fluorescence and " halo " even in the finished paper) from resin and general scum spots which are yellow and black, respectively (see also under Textiles, p. 368). Starch, shive, etc., are all characteristic, and coloured broke, which has been bleached, gives the paper a silurian effect in ultra-violet light. The *texture of paper*, or punctures in it, may be examined by smearing the underside with a mixture of vaseline and lubricating oil (1 : 1) and examining the top-side under the lamp, when every detail is shown vividly.[45]

Loadings as such are discussed under raw materials, but loadings in the paper are dealt with by A. F. Kitching [2] who endeavoured to link up the appearance of the paper with the nature of the loading and pulp process used, and by H. Ress [30] who confirmed the generally-accepted opinion that in most cases there is no relation between the colour of the fluorescence and that of the loading. He noted, however, that an increase in loading corresponds with an increase in brightness, and that as a result of this, the top-side of a sheet appears brighter than the under-side. This may be used to distinguish the two sides and as a means of investigating the distribution of the loading, although one of us (J. G.[45]) has found certain cases to which it does not apply, presumably because the fluorescence of the paper is brighter than that of the loading. J. F. Smith [46] confirms this, and has found that the

difference may be produced in newsprint by unequal distribution of the "flour" from sulphite pulp. Fluorescent substances have been suggested as indicators for the identification of coated papers.[55]

Sizing.—Rosin sizing produces a definite increase in the brightness of finished paper and as Photograph No. 6 shows, many wax sizes have a similar or even greater effect. One of us (J. G.[35]) has, however, been able to use the lamp to obtain a highly sensitive sizing test, the procedure being an adaptation of his method (p. 227) for the detection of artificial watermarks.

Sizing value is usually measured in terms of the resistance of the paper to penetration by water, and although there are about forty methods of estimating this, none are free from criticisms, the most important being the slowness with which the end-point becomes apparent; this may involve an error of over 15 per cent., and is as high as 10 per cent. even for the American "official" dry-indicator method. In the present test an almost instantaneous end-point, a penetration-period sufficiently rapid to eliminate false results due to local variations in the paper, and an error of less than 5 per cent. are obtained as follows:—

A mixture of 0·5 grm. of Rhodamine-6G (conc.) and 100 grms. of oven-dry icing-sugar is prepared by grinding in a mortar, and is stored in a small bottle (in the metal screw-cap of which is inset a piece of 70-mesh bronze gauze) in a desiccator. The powder is sprinkled over a 2-inch square of the specimen under test, which is then floated on the surface of distilled water at 21° C., and the time taken. The penetration of the water is indicated by the vivid golden glow suddenly produced in ultra-violet light against the dark background when the dye dissolves, and the time taken can be estimated with an error of little more than 2 seconds.

Mineral oil may be used in a similar way for testing grease-proofness, or if the paper is to be used in contact with a non-fluorescent oil or fat, the resistance to this may be examined by adding a fluorescent material to it (Grant [45]). M. Déribéré [63] has introduced refinements into this technique, notably the application of the water under a controlled hydrostatic pressure. The sample is clamped over the top of a small jar, the bottom of which has a side-arm, which is connected with a vertical graduated tube. If mercury is used in the tube and water in the jar, the

test may be accelerated by the pressure developed by the mercury, and the method is then very suitable for materials of high water-resistance. "Creeping" at the edges of the sample is minimised by the application of paraffin wax. Dériběré has also applied the method to the measurement of the permeability and water-resistance of materials other than paper (*e.g.*, see Chapter XVII, References).

In the version of Grant's method proposed by A. Noll and K. Preiss [65] a disc of the sample of paper (60 mm. in diameter) is held in an aluminium funnel, which is immersed to a constant depth in distilled water so that the water touches the underside of the paper under constant conditions of pressure. Good results are obtained if calcined sodium carbonate containing 0·001 per cent. of fluorescein is used as the indicator.

Tub-Sizing with gelatine dulls the brightness of the fluorescence of paper considerably, but many of the modified starches have just the reverse effect, and even in cases where the untreated paper is not available for comparison, it is frequently possible to say which method has been used; this is important, since the iodine reaction does not distinguish between starch used in this way and in the beater. Examination of a section of the paper under the fluorescence microscope has also been found helpful in this connection by Grant.[45]

The examination of finished papers from the legal and antiquarian points of view is discussed by E. W. Stein [38] and on pages 225 and 278; watermarks [45] are also dealt with.

Fading, and Fading- and Exposure-Tests.[48-50]—One of us (J. G.[36, 45]) and also K. Berndt [37] have carried out a number of fading tests on various papers with the object of correlating, if possible, the effects of sunlight and those of unfiltered ultra-violet light. It was found that, on the whole, the latter gave a fairly good qualitative indication of the former, *i.e.*, the rate of fading of a particular paper was about the same in both cases. The nature of the fading, however, was by no means always identical; thus, a paper dyed with two yellow dyes turned green in sunlight but red in ultra-violet light, and cream- and pink-tinted papers became lighter and darker, respectively; in some cases the relative stabilities of two papers as determined in sunlight was even reversed in ultra-violet light. Azures, on the other hand, were more consistent in behaviour. Since 30 minutes of

exposure to the mercury lamp are equivalent to about 1 week of bright north daylight, the test may be used with great advantage (so long as the above considerations are borne in mind) to forecast the extent, as distinct from the nature of the fading in sunlight, and for routine tests on specimens of known history. J. Grant,[36] S. Oguri [68] and others [67] have also attempted to follow the changes in chemical nature which occur during the exposure of cellulosic materials to ultra-violet light.

It is considered in most quarters, however, that the carbon arc simulates more closely the fading effect of sunlight, and suitable apparatus and procedure is described fully in a Report of the Society of Dyers and Colourists [39]; Fig. 10 on page 28 indicates the similarity of the spectra of sunlight and the carbon arc, and the intense lines just beyond the visible region (which are largely responsible for fading) will be noted. The general question of the suitability of various lamps for fading is outside our present scope, but is dealt with more fully elsewhere by J. Grant.[67]

In the course of these tests the interesting fact was noted that the fluorescence of the dyed paper itself changed during exposure, a phenomenon which might be expected, at any rate in cases where dyed papers were under consideration (see Photograph No. 6, facing p. 400). The method of E. H. Riesenfeld and T. Hamburger [11] in which half the specimen is exposed and the other half covered by a metal plate and the time noted for the production of a definite change in fluorescence (as distinct from a change in visible light), was therefore tested. It was found to give results having about the same order of accuracy as the method of direct exposure to the unfiltered rays described above. About 70 per cent. of the dyed papers tested gave results in agreement with those obtained in daylight, but the great advantage of the method rests in the fact that with fugitive dyes the result is obtained very rapidly and at any time of the day or year, since a change in fluorescence may be produced after a few minutes, although no corresponding change is necessarily visible in ordinary light. Here again, therefore, the method is of value for routine tests on known materials, as well of course for the identification of the dyes. On account of the difficulty of deciding when a certain degree of change in fluorescence has occurred, J. Grant [67] has found it more accurate to conclude the test when the first visible change in fluorescence is apparent, and to expose the

sample to daylight, rather than to ultra-violet light. In this way, the test serves in many cases (but subject to the limitations indicated above) to magnify the effects produced by exposure to the type of fading agent it will encounter in practice. In many cases results may be obtained very rapidly.

Where more time is available, however, it is more accurate to expose the papers to daylight, and to note the minimum time to produce a change in appearance in ultra-violet light [45]; this will not necessarily be accompanied by a change in appearance in visible light, and of course it will depend to some extent on the "quality" of the daylight.

Mention has frequently been made of the change in fluorescence which wood pulp itself undergoes after exposure to heat or light, and since such changes also occur in the finished paper, this must be taken into account in tests of the above type by examining the dye and paper separately. This of course applies particularly to papers coloured with fast dyes or with pigments, in which cases the paper itself changes before the colouring matter. Although it is only incidental to the present subject, it should be noted in the above connection that certain colouring matters, and in particular certain red, orange, and yellow vat dyestuffs, catalyse the destruction of cellulose on exposure to ultra-violet light. R. Haller and L. Wyszewianski [60] have investigated the conditions under which this occurs, and they also note that in some cases, the fluorescence increases in intensity when the dyeing is heated. Another precaution to be observed is to allow the lamp to run for about five minutes before exposing the specimen in order to eliminate the bleaching-effect of the ozone frequently produced when the lamp is started up. The work of S. Oguri [68] emphasises the importance of this precaution, because he showed that when ozone is excluded, degradation of the cellulose (as measured by the copper number) is decreased. The same paper contains data which show the relationship between exposure-time and copper number for various types of white papers in the dry and moist states.

Tub-Sized Papers (*i.e.*, good quality papers which contain less than the normal amount of rosin sizing, but are passed through a bath of gelatine when made). On prolonged exposure to sunlight such papers change to a brownish shade and to some extent lose their sizing effect. This was found to be accompanied

by a diminution of gelatine content, as shown both by the tannic acid and oil tests, and by a fall in *p*H value.

A note by H. A. Bromley [21] confirms that similar changes occur after exposure to ultra-violet light for a few hours. In this case the *p*H value fell from 6·6 to 6·1, but whatever reactions were taking place could not proceed to completion on account of the absorption of the rays by the degraded gelatine formed. It is probable that a ray having a wave-length common to both sources is responsible for this action which, apparently, does not result in the formation of the usual degradation products of gelatine. The photolytic degradation of gelatine is in fact a well-known phenomenon, and has been the subject of many studies, *e.g.*, by P. Ponthus [40] who related it to a decrease in the viscosity, and by Liesegang (*infra*).

In the case of paper exposed to tropical conditions, the question of heat as well as light is involved, and it is important to distinguish these effects so far as possible, because papers are usually stored in the dark although not necessarily in a cool place. It has been found by Grant [36] and by others, that both heat and light produce a similar type of degradation of the cellulose, which may be reduced to a minimum by ensuring that the paper is as free as possible from non-cellulosic impurities (*e.g.*, acids, etc.). Further, in general, gelatine tub-sizing protects the paper from light by the absorption which occurs when it becomes discoloured; starch on the other hand discolours more under the action of heat than of light.

Parchments and Transparent and other Speciality Papers.—A. von Schlitter [41] used the method to test transparent papers, and H. Sommer and J. Becker [42] found that in such cases also, the normal fluorescence changes, and eventually disappears, on exposure; the absorption of light was measured by impregnating the material with a fluorescent dye, the emission of which was measured by a Pulfrich photometer after varying periods of irradiation.

H. Salvaterra and F. Noss [43] examined sections of various papers in paraffin wax under the fluorescence microscope. True parchment appeared light blue, orange or maize with orange borders and yellow veins, according to the source of the paper and its manufacturing history. Greaseproof was a lighter blue with deeper veins parallel to the surface. Imitation parchments were always violet if made from unbleached pulp, and the structure

of the sheet (*e.g.*, whether triplex or not), of its surface, and of the individual fibres were plainly visible; this applies particularly to chromo-papers. The method is considered a valuable adjunct to ordinary microscopy in the differentiation of these various grades of paper. D. R. Morey [44] examined cellulose fibres which had been treated with a fluorescent dye, and was then able to relate the polarisation of the fluorescence with the micellar orientation and spiral structure of the fibres.

Imperfections in condenser papers are rendered visible, according to K. Stantien,[64] by examining the sample in ultra-violet light after it has been treated in succession with (*a*) a bath containing quinine in 1 per cent. sulphuric acid; and after drying, with (*b*) molten paraffin wax containing 1 per cent. of pyridine.

Opacity.—J. Grant [45, 52] has found that this method is also very useful to determine whether the lack of opacity of a paper is due to the paper itself, or to the penetration of the oil used as the ink medium under the printed letters. In the latter case, the degree of penetration of the oil through the paper is shown by a fluorescent glow; if the oil is not itself fluorescent a test may be made with this same oil to which a small quantity of a fluorescent substance has been added. With newsprint it is frequently possible to read one side of the page from the opposite side by means of the fluorescence of the penetrating oil, and an excellent photographic illustration of such a case is shown in a publication of the Printing and Allied Trades Research Association (Patra).[56] It is possible in this way, by studying sections of printed paper under the fluorescence microscope, to study the path of ink through the paper (see Photographs 24 and 25, p. 400). Fluorescence analysis has also been used [69] as an aid to the investigation of set off "by evaporation."

Cellulose and its Derivatives.—Cellulose has a strong fluorescence, and the mono-acetate fluoresces still more strongly, although, strangely enough, the higher acetates show a noticeable decrease in the intensity of the fluorescence, and nitrated cellulose and lignocellulose are almost non-fluorescent. Various wools and linens show fluorescence effects, but parchmentisation alters these to various extents, in some cases strengthening, and in other cases weakening the intensity. Powdered sugars were examined in layers by S. Judd Lewis [5, 6] in a similar manner, and

fluorescence curves were obtained which are interesting from a theoretical point of view (*cf.* p. 175).

The fluorescence of cellulose and its acetates and nitrate, and also of gelatine, has been examined by J. G. McNally and W. Vanselow,[12] who confirm the findings of Lewis, *viz.*, that the intensity of the fluorescence depends on the wave-length of the exciting light and on the chemical constitution. They consider further, that it is related to the micellar structure of the film. The intensity of the fluorescence of solutions of cellulose acetate

TABLE 27.

FLUORESCENCE OF YELLOW FILM WASTE BEFORE AND AFTER EIGHT HOURS OF EXPOSURE UNDER THE QUARTZ MERCURY-VAPOUR LAMP.

Softening Agent Used.	Fluorescence Before Exposure.	Fluorescence After Exposure.
Castor oil	Bright brown	Dark grey-green
Tricresylphosphate	Yellow-brown	Greenish-grey
Dibutylphthalate	Yellow-brown	Ochre-yellow
Glycomonomethylphthalate	Brown	Ochre-yellow
Di-*iso*-butylphthalate	Yellow-brown	Grey-green
Dimethylphthalate	Yellow-brown	Grey-beige
Glycerindicresol ether	Bright brown	Dark grey-brown
Diamylphthalate	Dull yellow-brown	Dark grey-brown
p-Toluolcresylsulphonate	Dull yellow	Dark grey-olive
p-Toluolethylsulphonate	Bright brown	Olive-grey
Ethylacetanilide	Dull yellow	Dark grey
Triphenylphosphate	Grey-brown	Dirty grey-olive
Monoethyltoluolsulphamide	Dull yellow-brown	Greenish-grey
Diethylphthalate	Ochre-yellow	Grey-olive
Methylcyclohexyl ester of methyladipic acid	Grey-brown	Blue-grey
Iso-butyltartrate	Ochre-yellow	Dark grey-brown

in acetone appeared to be independent of the colloidal properties of the solution, but with gelatine a decrease in the viscosity of the solution produced a decrease in the intensity of the fluorescence (*cf.* p. 52).

Liesegang[19] mentions the fluorescence of gelatine, cellulose and collodion films in ultra-violet light, and also states that on exposure to sunlight for some time this fluorescence disappears or else changes in colour (*vide supra*). The invisible structural changes which occur in nitrocellulose films on exposure to light become strikingly evident from the alteration in the fluorescence colour, and this fact has been applied by W. Mager[20] and by

E. Beutel and A. Kutzelnigg [66] to the investigation of films used in the artificial leather and allied industries. In this connection, attention should be drawn to a new type of lamp (due to W. Münzinger [62]) for exposure tests on nitrocellulose products, the range of which includes the visible, infra-red and ultra-violet regions.

TABLE 28.

FLUORESCENCE OF COLLODION PYROXYLIN SURFACE BEFORE AND AFTER EIGHT HOURS OF EXPOSURE TO THE QUARTZ MERCURY-VAPOUR LAMP.

Softening Agent Used.	Appearance in Daylight After Exposure.	Fluorescence Before Exposure.	Fluorescence After Exposure.
Castor oil	Weak yellow	Bright blue	Luminous Yellow-green
Tricresylphosphate	Brown	Grey-violet	Brown-olive
Dibutylphthalate	No change	Neutral grey	Greenish-grey
Glycomonomethylphthalate	No change	Grey	Greenish-grey
Glycomonoethylphthalate	No change	Grey	Yellowish-grey
Di-*iso*-butylphthalate	No change	Grey	Greenish-grey
Dimethylphthalate	No change	Grey	Dark grey
Glycerindicresol ether	Yellowish-brown	Bright Neutral grey	Olive
Diamylphthalate	Yellow	Bright blue	Dark olive
p-Toluolcresylsulphonate	Faint yellow	Bright grey	Greenish-brown
p-Toluolethylsulphonate	Brown	Neutral grey	Yellowish-green
Ethylacetanilide	Brown	Grey-violet	Dark brown
Triphenylphosphate	Strong brown	Grey-violet	Brown-olive
Monoethyltoluolsulphamide	No change	Blue-grey	Bright Yellow-green
Diethylphthalate	No change	Grey-violet	Greenish-grey
Methylcyclohexyl ester of methyladipic acid	No change	Grey-blue	Greenish-grey
Iso-butyltartrate	Brown	Violet	Bright brown
Lactanilide	Brown	Grey	Brown
p-Toluolsulphamide	Brown	Bright violet	Dull olive
p-Toluolsulphanilide	Brown	Dull Yellow-green	Brown, Almost black

If a piece of art leather is examined under the lamp before and after exposure to sunlight, or to unfiltered light from a mercury arc, the fluorescence is found to have changed in colour (*cf.* gelatine, *supra*), and the extent of the change appears to bear some relation to the softening agent employed and to the changes which the latter undergoes. Mager gives two tables showing the behaviour of nitrocellulose films, made from various softening

agents (see also Göthel [29]) but containing no colouring matter, and deposited on fabric. Although the fluorescence of the coloured fabric itself has some influence on the colour obtained, the differences due to the various softening agents are sufficiently pronounced to be seen clearly. Table 27 shows the fluorescence observed when the film is made from waste pieces of yellow celluloid on a grey fabric base.

Table 28 gives for comparison the fluorescence colours of an uncoloured collodion pyroxylin film on the same material, the differences from the first table being therefore due to the camphor and dyestuff present in the film waste used in the former case. In all instances the colour of the fluorescence before exposure was affected by the grey dye in the fabric used.

Elm's [58] deals with the fluorescence of pigments used in plastics and varnishes, and an example of the use of ultra-violet light as an aid to the investigation of the structure of cellulose membranes is provided by the work of W. K. Farr,[61] who used these radiations and polarised light as sources for microscope examination.

REFERENCES.

[1] W. N. Hartley, *Jour. Chem. Soc.*, 1893, 245.
[2] A. F. Kitching, *Analyst*, 1922, 47, 206.
[3] S. Judd Lewis, *Jour. Soc. Dyers and Colourists*, 1918, 34, 167.
[4] S. Judd Lewis, *ibid.*, 1921, 37, 201.
[5] S. Judd Lewis, *ibid.*, 1922, 38, 68.
[6] S. Judd Lewis, *ibid.*, 1922, 38, 73.
[7] S. Judd Lewis, *ibid.*, 1924, 40, 29.
[8] S. Judd Lewis, *ibid.*, 1924, 40, 111.
[9] M. Fallot, *Chim. et Ind.*, 1928, 19, 596.
[10] H. Wislicenus, *Zeit. angew. Chem.*, 1928, 41, 1315.
[11] E. H. Riesenfeld and T. Hamburger, *Pap. Fabrik. (Tech. Wiss. Teil.)*, 1931, 29, 164.
[12] J. G. McNally and W. Vanselow, *Jour. Amer. Chem. Soc.*, 1930, 52, 3846.
[13] H. G. Klein, *Pap. Fabrik.*, 1930, 28, 477.
[14] K. Berndt, *Zellstoff u. Papier*, 1931, 11, 257.
[15] O. Gerngross, *Zeit. angew. Chem.*, 1926, 39, 1028; *Pap. Fabrik.*, 1927, 25, 49.
[16] P. Rassow and G. Brandau, *ibid.*, 1929, 27, 203.
[17] H. G. Klein, *ibid.*, 1930, 28, 477.
[18] H. G. Klein, *Zellstoff u. Papier*, 1931, 11, 81.
[19] R. E. Liesegang, *Zeit. wiss. Mikros.*, 1927, 43, 517.
[20] W. Mager, *British Industrial Finishing*, 1931, 2, 190.
[21] H. A. Bromley, *Analyst*, 1933, 58, 29.
[22] V. Gruenmann, *Le Papier*, 1932, 35, 531.
[23] F. V. Lutati, *Boll. Uff. Staz. Sperim. Ind. Pelli.*, 1933, 11, 436.

[24] V. Hottenroth, *Pap. Fabrik.*, 1934, **32**, 557.
[25] Kimreuther, Schulze and Nippe, *ibid.*, 1926, **24**, 106.
[26] F. Noss and H. Sadler, *ibid.*, 1932, **30**, 541 ; 1933, **31**, 413.
[27] B. Schulze and E. Göthel, *ibid.*, 1934, **32**, 110 ; *Zellstoff u. Papier*, 1934, **14**, 93 ; *Woch. Pap.*, 1934, No. 7.
[28] J. Grant, *Nature*, 1934, **134**, 635.
[29] E. Göthel, *Diss.*, Dresden, 1933.
[30] H. Ress, *Pap. Fabrik.*, 1934, **32**, 403, 409, 454.
[31] J. Grant, *Sands, Clays and Minerals*, 1934, **2**, 49.
[32] H. Pringsheim and O. Gerngross, *Ber.*, 1928, **61 B**, 2009.
[33] E. H. Riesenfeld and T. Hamburger, *Pap. Fabrik.*, 1929, **27**, 528.
[34] G. Testi, *Chem. Ind. Agr. Biol.*, 1933, **9**, 470.
[35] J. Grant, *Jour. Soc. Chem. Ind.*, 1934, **53**, 349T ; *Analyst*, 1934, **59**, 749.
[36] J. Grant, *Proc. Tech. Sec. Paper Makers' Assoc.*, 1934, **14**, 335.
[37] K. Berndt, *Zellstoff u. Papier*, 1933, **13**, 271.
[38] E. W. Stein, *Quartz Lamp*, 1932, **10**, 86.
[39] " Standards for Light, Perspiration and Washing " (Bradford, *Soc. of Dyers and Colourists*, 1934).
[40] P. Ponthus, *Compt. rend.*, 1933, **196**, 1248.
[41] A. von Schlitter, *Kunstseide*, 1933, **15**, 158.
[42] H. Sommer and J. Becker, *Zellstoff u. Papier*, 1933, **13**, 335.
[43] H. Salvaterra and F. Noss, *Pap. Fabrik.*, 1934, **32**, 371.
[44] D. R. Morey, *Textile Res.*, 1934, **4**, 491.
[45] J. Grant, *Proc. Tech. Sec. Paper Makers' Assoc.*, 1935, **16**, 97 ; *Paper Trade Rev.*, 1935, **103**, 1463.
[46] J. F. Smith, *ibid.*, 1935 (Discussion on above paper).
[47] B. Schulze, *Pap. Fabrik.*, 1935, **33**, 165.
[48] Smith, *Packaging Gaz.*, 1935, **12**, 340.
[49] Kupferath, *Woch. Pap.*, 1935, **66**, 163.
[50] Anon, *Proc. Tech. Sec. Paper Makers' Assoc.*, 1936, **17**, 9.
[51] J. Grant, " Wood Pulp " (Leiden : Chronica Botanica Co.), 1938.
[52] J. Grant, *Paint Manuf.*, 1937, **7**, 340.
[53] H. J. Henk, *Kunstseide u. Zellwolle*, 1937, **19**, 426.
[54] M. Déribéré, *Papier*, 1935, **38**, 797 ; " Applications de la Lumière de Wood et des Rayons Ultra-Violets dans les Industries Textile et Tinctoriale " (Paris, Editions Textile et Technique), 1937.
[55] Adhesives Gieseck and Devrient, A.G., G.P. 647,929, 1937.
[56] Anon, *Patra Jour.*, 1938, **1**, 143 ; G. M., *ibid.*, 1939, **2**, 175.
[57] B. Schulze, *Pap. Fabrik.*, 1937, **35**, 25, 37.
[58] Elm's, *Rév. Gén. Mat. Plast.*, 1938, **14**, 154.
[59] A. Noll, *Pap. Fabrik.*, 1938, **36**, 41.
[60] R. Haller and L. Wyszewianski, *Melliands Textilber.*, 1936, **17**, 45, 138.
[61] W. K. Farr, *Pulp and Paper Mag. Canada*, 1936, **37**, 74 ; *Jour. Appl. Phys.*, 1937, **8**, 228.
[62] W. Münzinger, *Nitrocellulose*, 1935, **6**, 179.
[63] M. Déribéré, *Papeterie*, 1935, **57**, 966.
[64] K. Stantien, G.P. 577,012, 14/7/28.
[65] A. Noll and K. Preiss, *Pap. Fabrik.*, 1937, **35**, 213.
[66] E. Beutel and A. Kutzelnigg, *Farbe und Lack.*, 1934, 351.
[67] J. Grant, " Books and Documents. Dating, Permanence and Preservation " (London : Grafton), 2nd Edn., 1939.
[68] S. Oguri, *J. Soc. Chem. Ind. Japan*, 1934, **37 B**, 620.
[69] J. H. Y., *Patra Jour.*, 1939, **2**, 225.

CHAPTER XVII.

THE RUBBER INDUSTRY.

THE use of ultra-violet light for routine testing in the rubber industry is developing rapidly. Such work falls into two classes: (*a*) Experiments on ageing by the production of those photochemical reactions which occur in the rubber on exposure to the lamp or to sunlight. (*b*) The examination of materials in filtered ultra-violet light, and the determination of their source and quality from the resulting fluorescence.

Ageing Tests.—The first class of work includes the ageing tests carried out on rubber goods, such as the cracking of motor tyre treads and sidewalls on exposure, and the fading of coloured rubber stock. According to Garner [1] all ingredients in rubber deteriorate in light, particularly if they are originally dark in colour, and rubber solutions decrease in viscosity. The criteria of deterioration are also discussed, and it is thence pointed out that opinions differ as to the value of ultra-violet light in accelerating those changes normally produced by sunlight. Thus Weightman [2] states that mercury-vapour lamps cannot wholly replace sunlight, whilst others consider that under standard conditions the former provide a reliable and comparable result. Evans,[3] and also Naunton and Defries [6] support the latter workers, and show the results of the former to be due to the fact that the differences between two results obtained in ultra-violet light are much smaller than those found in open exposure tests (see also Shepard and his co-workers,[4] Shacklock,[21] Somerville [5] and V. Margaritov [24]). Ageing tests were also carried out by B. Dogadin and V. Balandina,[16] and by V. N. Morris [17] who notes that the fluorescence of rubber changes on exposure to ultra-violet light.

Henri [19] has noted that raw rubber is affected to a greater extent than vulcanised rubber, and Asano records the fact that exposure renders rubber less soluble in many solvents. M. Krahl,[7] who worked on cable mixings, does not consider that

ozone is responsible for the changes produced, but Berstein [20] holds precisely the opposite view.

In summarising such work H. Barron [22] expresses the opinion that, if used in conjunction with the Geer oven-test, the oxygen bomb-test and, in the case of tyres, flexing-tests, the method provides a very good idea how a material will behave as a result of normal ageing.

Nagle [12] gives a test for the stability of *lithopones* to sunlight in which standard mixings of the sample with glycerin are exposed under the lamp. Defective samples should blacken in 30 seconds to 5 minutes, but reliable samples are unchanged after 15 minutes. In this connection it should be remembered that darkening occurs more rapidly in glycerin than in the actual rubber mix. Nagle also examined zinc oxide and found that various commercial samples, apparently identical in daylight, can be distinguished one from the other and may even be identified in mixings with carbon black (see also p. 200). With lithopones a rough gradation is obtained according to the fluorescence colour, which also indicates decreasing degrees of sun-proofness, as follows: 1. Deep violet, most stable. 2. Pale violet, less stable. 3. Brown. 4. White, least stable to sunlight.

So far as concerns *surface-vulcanisation* of unvulcanised rubber on exposure to light, and darkening due to local *overcure*, Naunton and Defries [6] have shown that ultra-violet light can simulate sunlight, although emphasis is laid on the necessity for standardisation of the methods used. The humidity of the atmosphere appears to be an important factor, and this has also been found to be the case in the textile-dyeing, paper, and paint and varnish industries. Although this work, strictly speaking, is outside the province of this book, it is mentioned because it provides examples of the use of the lamp for testing purposes and for technical control (*cf.* pp. 344 and 353).

Examination of Materials.—The second class of work with the lamp is that in which we are more interested, namely, the examination of raw and other stuffs in filtered ultra-violet light. The examination of rubber itself cannot be materially facilitated in this way, but certain *loading substances*, compounds such as zinc oxide or lithopone, show a strong fluorescence and may be detected in various mixtures (*cf.* p. 342). The lamp therefore provides a simple method for the determination of the identity

of a number of compounds and is often of great help in assessing their purity. A very full description of the ultra-violet microscope is given by F. F. Lucas,[26, 28-30] who has applied it to the study of latex. He finds that approximately 90 per cent. of the particles are 0·50 micron or less in diameter, and that their shape is predominantly spherical. The use of the ultra-violet microscope is assisted by photography, as the images seen in the fluorescent eyepiece are only faintly visible. By the use of this apparatus, however, a greatly increased resolving power is obtained, some 250,000 lines per in. being realised as against the 140,000 lines per in. obtained with a homogeneous immersion objective of N.A.I. 40. Green[27] has also used this method to examine vulcanised latex. M. Krahl[7] has examined a number of *rubber mixes* in the raw, unvulcanised and vulcanised states and also a number of the other compounds used in the industry. Impurities in raw materials are often detectable, *e.g.*, oils show a bright fluorescence. The ashes of some rubbers show a strong fluorescence, and in some cases it is possible to detect the omission or presence of a particular ingredient in the rubber mixing, although this is generally a matter of difficulty.

Pigments give the colours shown in Table 29 when examined under the lamp, and are further discussed under Paints (p. 329) and Paper (p. 340).

TABLE 29.

Lithopone.	Green-yellow.	Calcined magnesia.	Bluish-green.
Zinc sulphide.	Bright orange.	Magnesium carbonate.	Violet.
Lime.	Light brown.	Titanium white.	Deep brown.

The above observations are those of Garner,[8] but it must be remembered that sometimes the mode of preparation or the source of a substance alters the shade of its fluorescence colour. As F. Kirchhof[9, 10] has pointed out, such differences in various commercial preparations depend largely on differences in grinding and purity, and this is true especially for substances such as zinc oxide, the purest varieties of which glow most strongly under the lamp (*cf.* p. 331). Similarly, the progress of the mastication process may be followed from the corresponding change in appearance of the zinc oxide present[18] (*e.g.*, under the microscope).

Garner has also examined the fluorescence of pigments under the microscope after staining with a fluorescent dye. Magnesium carbonate may be treated in this way with a solution of eosin in ether, when the weakly-calcined particles give a beautiful fluorescence which is not shown by those which have been more strongly heated. V. N. Morris [17] finds that various commercial brands of zinc oxide are distinguishable even in the ash from the rubber, so long as the incineration is carried out at a low temperature and due allowance is made for variations in particle-size.

The organic compounds (*accelerators, etc.*) tested showed colours as follows :—

Vulkazit-*CA*.	Weak yellow.
Vulkazit-*D* (Diphenyl guanidine).	Reddish-violet.
Vulkazit-1000 (*o*-toluyl guanidine).	Strong bluish-violet.
Stearic acid.	Pale blue-violet.

The sulphur-compounds Vulkazit-*P* and Thiuram give the bright brown fluorescence shown also by piperidine and similar to that of sulphur, whilst Captax (mercaptobenzothiazole) appears orange. Some *antioxidants* also fluoresce characteristically ; for example, Agerite in the massive state gives a bluish-green colour, although in benzol solution the fluorescence is violet. K. Kojima and I. Nagai [25] examined a comprehensive range of 41 different accelerators, and found that most of them fluoresce in a characteristic manner.

V. N. Morris [17] has also tested many materials used in the rubber industry and some of his results with softeners are shown below :—

Pine tar.	Dark yellow-green.
Refined asphalt.	Dark yellow-brown.
Mineral rubber.	Purple (nearly black).
Rosin oil.	Light blue.
Rosin.	Intense light blue.

He considers that the method is an aid to the identification of softeners extracted from products of unknown composition.

With anti-oxidants the following results were obtained :—

Acetaldehyde aniline.	Dull greenish-brown.
N . N′-Diphenylenediamine.	Red violet.
Hydroquinone.	Blue violet.
Flectol.	Greenish-yellow.
Phenyl β-naphthylamine (sample A).	Luminous light blue.
Phenyl β-naphthylamine (sample B).	Luminous light blue-violet.
Phenyl α-naphthylamine (sample A).	Luminous light blue.
Phenyl α-naphthylamine (sample B).	Luminous violet.

It will be noted that compounds of similar compositions, but from different sources, show slight variations in fluorescence colour.

Of the accelerators examined, the majority showed tones of purple (9 samples), others were completely non-fluorescent and non-reflective and appeared black (5 samples), whilst the appearance of some of the others is given below :—

Ethylidene aniline.	Light brown.
Trimene base.	Dark yellowish-green.
Trimene.	Light green.
Captax.	Reddish-brown.

Morris finds further, that well-vulcanised rubber has a fairly strong yellow fluorescence, whilst decidedly under-cured rubber fluoresces only weakly. In general no definite relationship was obtained connecting the optimum physical properties of the rubber with its fluorescence. Exposure of rubber to sunlight for about an hour destroyed the fluorescence.

Further work on the fluorescence of *protective agents* and accelerators has been done by R. Ditmar,[11] and his observations substantially confirm those already described. This method is considered by Ditmar to be of use in the control of commercial grades, since the colour given by one particular product, *e.g.*, mercaptobenzothiazole, may vary according to its source or manufacturer.

Ditmar and Dietsch [13, 14] also applied this method of examination to the rubber dust from thin-walled, sulphur chloride-vulcanised rubber articles. They attempted to distinguish between the use of different accelerators, and used a number of known vulcanisation accelerators alone, and also in admixture with clear Hevea-crepe raw rubber (free from impurities such as sulphur). The accelerators *A*-Bayer, *TR*-Bayer, 470-Bayer and accelerator-*BB* each gave a characteristic fluorescence to such mixtures. The nature of the fluorescence may vary for one accelerator according to the nature of the vulcanisate, but it is usually characteristic and is maintained even in the presence of sulphur, zinc oxide, lead oxide or magnesium oxide, etc. If many fluorescent compounds are present at once it is possible to assess them by fluorescence methods, but only by comparison with similar mixtures of known compositions.

Rubber materials usually fluoresce blue-grey to violet-grey,

but certain additions, or contamination with substances such as ceresin or vaseline, modify this fluorescence and may thus be recognised. In some cases pre-extraction with acetone is useful. H. Barron [22] confirmed the results of Morris as a whole, and some of his observations are given in the following table :—

Normal latex.	Light purple.
Revertex.	Bright white.
Vultex.	Greenish-white.
Colloidal sulphur.	Light purple.
Casein.	Bright yellow.
Dextrin.	Bright brown.
Turkey red oil.	Bright white.
Triethanolamine.	Dull green.
Saponin.	Brilliant white.
Sodium silicate.	Light green.
Sulphonated lorol.	Brilliant white.
Vulcastab A.	Bright white.

He suggests that the lamp might be used for the routine examination of ingredients before mixing, especially in conjunction with capillary analysis. Although Cotton [23] reports an increase in the intensity of fluorescence with an increase in tensile strength, and although Morris [17] has claimed that well-vulcanised rubber can be distinguished from under-cured rubber by its yellow fluorescence, the results are not conclusive, and Barron considers that in its present state the method is unsuitable as a means of evaluating the state of cure of a mix.

The examination of rubbers and vulcanising agents has also been carried out by Lange,[15] but as he used unfiltered ultra-violet light, his work will not be considered further. The uses of the lamp in the rubber industry have been summarised by Kirchhof,[9, 10] and according to him it may be used (*a*) for the rapid qualitative testing of all kinds of substances added to the mix, *i.e.*, to distinguish one from another and to test for purity ; (*b*) estimates of quantities may also be obtained by comparison with standard mixtures containing known quantities of the substances investigated ; (*c*) changes due to natural or artificial oxidation (*e.g.*, ageing) may be followed systematically.

REFERENCES.

[1] F. L. Garner, *Industrial Chemist*, 1931, 7, 395 ; 1935, 11, 177 ; *Ind. Rubber Inst. Trans.*, 1928, 3, 417 ; 1929, 4, 413.

[2] Weightman, *Rubber Age* (N.Y.), 1928, 23, 75.

[3] B. B. Evans, *Ind. Rubber Inst. Trans.*, 1930, 5, 442.

[4] N. A. Shepard, S. Krall and Morris, *Bur. of Stds. Tech. Paper*, 1927, No. 3425.

[5] Somerville, *Rubber Age*, 1929, **10**, 363.

[6] W. J. S. Naunton and Defries, *Ind. Rubber Inst. Trans.*, 1929, **5**, 299.

[7] M. Krahl, *Kautschuk*, 1927, **3**, 159, 181.

[8] F. L. Garner (British Hanovia Quartz Lamp Co., Ltd., trade pamphlet), "Fluorescence Analysis, instant test of raw materials for grade and genuineness," p. 15.

[9] F. Kirchhof, *Kautschuk*, 1927, **3**, 317.

[10] F. Kirchhof, *ibid.*, 1928, **4**, 24.

[11] R. Ditmar, *Caoutchouc et Gutta-Percha*, 1931, **28**, 15, 685.

[12] P. G. Nagle, *Ind. Rubber Inst. Trans.*, 1927, **3**, 304; *Rubber Chem. and Tech.*, 1928, **1**, 284.

[13] R. Ditmar and W. Dietsch, *Gummi Ztg.*, 1928, **42**, 1415.

[14] R. Ditmar and W. Dietsch, *Chem. Ztg.*, 1928, **52**, 388.

[15] Lange, *Gummi Ztg.*, 1926, No. 8.

[16] B. Dogadin and V. Balandina, *Kautschuk*, 1933, **9**, 146.

[17] V. N. Morris, *Jour. Ind. Eng. Chem.*, 1934, **26**, 107.

[18] E. A. Hauser and S. Le Beau, *Kautschuk*, 1934, **10**, 113.

[19] Henri, *ibid.*, 927, **5**, 159.

[20] Berstein, *Koll. Zeit.*, 1913, **12**, 193.

[21] Shacklock, *Ind. Rubber Inst. Trans.*, 1930, **6**, 262.

[22] H. Barron, *Rubber Age*, 1934, **15**, 219.

[23] F. H. Cotton, *ibid.*, 1931, **12**, 307.

[24] V. Margaritov, *Jour. Rubber Ind. U.S.S.R.*, 1936; *Kautschuk*, 1936, **12**, 94.

[25] K. Kojima and I. Nagai, *Jour. Rubber Soc. Japan*, 1930, **2**, 260.

[26] F. F. Lucas, *Ind. Eng. Chem.*, 1938, **30**, 146.

[27] H. Green, *ibid.*, 1925, **17**, 802.

[28] F. F. Lucas, *Amer. Inst. Min. Met. Engrs.*, Pamphlet 1576-E, 1926.

[29] F. F. Lucas, *Proc. Nat. Acad. Sci.*, 1930, **16**.

[30] F. F. Lucas, *Jour. Franklin Inst.*, 1934, **217**, June.

ADDITIONAL REFERENCES.

A. S. Karsten, *Caout. et Gutta-Percha*, 1935, **32**, 7151; *Kautschuk*, 1935, **11**, 1078 (General).

M. Déribéré, *Caout. et Gutta-Percha*, 1936, **33**, 17, 656, 657 (Application of Grant's sizing test, *cf.* p. 343, to penetration of liquids other than water).

Anon, *Rubber Age* (N.Y.), 1936, **39**, 96 (General).

J. Grant, *Brit. Trade Jour. and Export World, Plantations Suppl.*, 1937, **75**, XXV (General).

J. A. Radley, *Electrical Times*, 1936, Feb. 13 (Examination of materials for the electrical industries).

Anon, *Gummi Ztg.*, 1937, **51**, 1100; H. G. G. Desmarest, F.P. 786,572 (Stable luminous pigments for use in rubber cloth).

CHAPTER XVIII.

TEXTILES.

IN the textile industry, as in the paint industry, the quartz mercury-vapour lamp is largely used to carry out *exposure and fading tests*, but a full consideration of this branch does not come within the scope of this book (*cf.* pp. 324 and 344). Reference should, however, be made to the fuller discussions given in connection with paper and paints (since similar considerations apply on the whole as to textiles), to the Report of the Society of Dyers and Colourists,[28] in which a carbon arc is recommended, and to the work of the corresponding body in America,[43] on comparisons of the various types of lamp.

L. Meunier and G. Rey [23] mention the fact that the fluorescence of wool gradually disappears on prolonged exposure to ultra-violet light, and this appears to be a property shared by a number of substances to which reference is made in various other sections (see especially Paper). W. Weltzien [24] has examined the suitability of various types of lamps for this class of work and further general information may be obtained from the references.[34, 35, 40] The fluorescence of the various materials, however, also provides valuable information, and it is perhaps worthy of mention that the lamp should be of the large hanging type in order to deal with a greater area than usual. When the fabric is only slightly fluorescent, the fluorescence may be influenced considerably by the transmission characteristics of the filter used, so that in reporting results, the nature of the latter should always be specified.

Textile fabrics show great differences in the intensity and colour of their fluorescence, but the value of the results is somewhat lessened by the fact that *dressing materials*, oils and dyestuffs often completely alter the colour of the fluorescence [42]; on the other hand, as Q. Hirsch [37] has indicated in the case of diastase preparations, the brilliant and characteristic fluorescence of these may actually be of assistance in analysis. Reliable

results are, however, obtained from raw, undyed fibres, and these, according to H. Sommer,[1, 2] do not show very great differences in fluorescence colour. *Animal fibres* may be differentiated from those of vegetable origin under the lamp, bleached cotton can be distinguished from bleached flax, and cotton and unbleached flax from hemp. Genuine camel-hair fluoresces with a reddish-grey-brown colour and is easily distinguished from coloured wool, while genuine makes can be distinguished from the coloured cotton often used as an imitation.

In general, vegetable fibres show a yellowish fluorescence which after the usual treatments to which textiles are subjected, *i.e.*, scouring, bleaching, etc., changes to dull tones of blue and violet.

Physical differences in cellulosic fibres appear to be without any marked effect on the fluorescence shown. Impurities in the cotton, such as dirt, which affect its colour and appearance in daylight play an important part by influencing its appearance under the lamp and in the presence of dyestuffs this appearance can be modified considerably. A dark American grade of cotton can, therefore, appear almost indistinguishable from a light Egyptian Mako, although imitation Mako can be distinguished from the genuine product.

The methods of fluorescence microscopy (p. 78) may conveniently be applied to textile fibres, and reference should be made in this connection to the results obtained with paper fibres (p. 338). E. Grünsteidl [27] and R. Lassé [36] record work on such lines, the primary fluorescence colours of raw cotton and of raw and bleached mercerised cottons being bright blue, dull grey and blue-grey, respectively. Fluorescent dyes may be used to obtain a secondary fluorescence (see J. Grant [51]), or the specimen may be saturated with a 0·5 per cent. solution of quinosol (*o*-hydroxy quinoline sulphate), washed, dipped in 5 per cent. sodium hydroxide solution, and observed in ultra-violet light. Cotton produces a violet colour and linen a striking canary yellow, the colour being accentuated in the presence of zinc salts.

Cotton.—The examination of cotton is best carried out on a carded yarn in which the fibres have been smoothed and lay parallel in the sample. The colours of raw cottons from different sources will be found to range from white with a yellow tinge, to a decided brown with purple reflections.

Some of the results of W. H. Ellinger and L. Windschauer [17, 18] and of T. H. Daugherty and E. V. Hjort [41] are reproduced in Table 30. The latter workers used the fluorescence microscope, and although the data given are for undyed and unmordanted fibres, they found that lightly-bleached-and-dyed fibres gave substantially the same results; the microscopic method enables the constituents of fibre mixtures to be picked out, whereas to the unaided eye only a single composite colour effect is seen.

TABLE 30.

Fibre.	*Colour of Fluorescence.*
Bleached wool (zephyr yarn).	Clear bluish-white.
Carding wool (white raw, No. 15).	Bluish with distinct tendency to yellow.
Natural silk (scoured and degreased).	Clear blue; [30] some raw silks have a buff colour, paler than viscose rayons and brighter than nitrate rayons.
Unbleached wool (tops).	Clear blue.
Sthenosised rayon (Rayon treated with formaldehyde).	Clear golden.
Cuprammonium rayon.	White with purple shadings; brighter than acetate rayons.
Cellulose acetate.	Bluish-violet, or dull white; less bright than cuprammonium rayon.
Bemberg silk.	Reddish-white with strong blue-violet tints.
Viscose rayon.	Sulphur-yellow, with violet "shadows"; less bright than raw silk.
Nitro rayon.	Flesh colour, without brown shadings.
Mercerised and bleached cotton.	Clear golden.
Raw American cotton (1st quality 30's warp yarn).	Pale gold with a tinge of white.
Egyptian cotton, untreated.	Pale brown.
Unbleached, mercerised Egyptian cotton.	Very dark violet.
Egyptian cotton boiled with soda.	Dark brown, with violet patches.

Nopitsch [15] holds the opinion that for the differentiation of individual grades of cotton the lamp method offers no advantages over direct observation in daylight, and it should also again be noted that it is not possible to apply the former to dyed materials unless all traces of dyes and mordants are removed, as erroneous results may otherwise follow. The influence of mordants may be considerable (see Photograph No. 10, facing p. 400).

The treatment which a cotton has received often affects its fluorescence; thus, boiling with alkali dulls the fluorescence colour, and this change is most marked with mercerised, unbleached cotton. Nopitsch [14] considers that the brightness and shade of the fluorescence colour in this case are particularly dependent on the degree of stretching in mercerising, as this causes a loss in brightness and a tendency of the colour tone to move towards the violet region.

Bleached cotton, especially if glossed, appears duller than raw yarns, but on mercerisation the fluorescence is again increased although its shade is different.

A number of workers [4, 5] have used the lamp to detect adulteration of kapok, and to differentiate old and used cotton material from fresh material to be used as stuffing in upholstery. The new material appears blue, whereas old material is generally ivory white in colour, under the lamp. S. Moskowitz and his co-workers [7] point out, however, that used cotton fillings generally have a sulphur dioxide content of over 0·25 per cent., and show an ivory or tan-coloured fluorescence; the new fillings, however, appear blue, except when they have been fumigated with sulphur dioxide or loaded with sulphur compounds (*e.g.*, from certain clays), or have been exposed to air saturated with moisture for over six weeks, when the new fillings then show the ivory fluorescence similar to that of the old fillings.

In Maryland (U.S.A.) the law requires that second-hand materials used for filling bedding and upholstery must be so labelled, and A. W. Winne and J. D. Donovan [5] have therefore investigated fully the possibilities of fluorescence analysis for testing purposes in such cases. It was found that as cotton ages the fluorescence turns from a violet to a brown-white colour, and that this change is probably associated with the oxidation of cellulose to oxycellulose. In the majority of cases the test is accurate and very convenient, but occasionally complicating factors arise, *e.g.*, with staple cottons or spinning mill wastes of the better grades, the fainter violet fluorescence of which may be confused with the white fluorescence of older materials. In such cases the method must be supplemented by other tests, *e.g.*, with Congo red or methylene blue.

E. Grünsteidl [8, 9] has published photomicrographs of raw cotton, which exhibits a light bluish-white fluorescence; raw

mercerised cotton, which shows a dull greyish yellow ; and bleached mercerised cotton, which has a bluish-grey fluorescence. He has employed the secondary fluorescence method for distinguishing between mercerised and unmercerised cotton, Eosin Extra-Yellow being used to impart an intense yellow fluorescence to the mercerised cotton although it leaves the raw cotton unchanged.

Other Cellulosic Fibres.—The retting of flax has also been examined by this worker in conjunction with O. Mecheels,[10] but the tests described cannot be applied to chemically retted fibres. These fibres appear a yellowish-grey colour under the lamp, and are much duller in appearance than raw cotton and cannot be distinguished from each other, or from jute, by fluorescence analysis.[14] Bleached linen yarn shows a yellowish-white fluorescence of similar intensity to that of mercerised cotton, and bleached linen fabric shows a much yellower fluorescence than bleached cotton fabric. Although jute cannot readily be distinguished by its fluorescence from the other yarns, the violet colour of many jutes disappears after extraction with petroleum spirit as mineral oil is used widely in the treatment of jute ; as this is not used for the other fibres mentioned, this test is more or less characteristic for jute.[14] Loose ramie also shows a yellow fluorescence, somewhat redder in shade than that of kapok.

Wool and Animal Fibres.—The agent responsible for the fluorescence of hair and wool is keratin, which forms the main constituent of the fibres. The presence of fats and dirt in raw wool exerts a strong masking action on the fluorescence, and with furs and hair the natural colour present plays a similar rôle to that of the dirt in wool. When these agents are removed by scouring or bleaching the fluorescence of the fibres is readily seen. The blue fluorescence of wool is increased by washing, and it thus acts in the opposite manner to cotton. One of us (J. A. Radley) has found that the action of small amounts of caustic soda tends to reduce the fluorescence of wool to a minimum value, after which the use of increased amounts of alkali appears to cause no further drop in intensity. Normal chlorination, using 1° Tw. sodium hypochlorite solution, reduces the intensity of fluorescence a little whilst treatment with a 5° Tw. solution results in a very definite decrease. On boiling wool for

15 minutes with 5 per cent. sulphuric acid, the fluorescence increases slightly, while treatment with 10 and 20 per cent. acid under the same conditions causes the tone of the fluorescence to redden. Carbonised wool is less fluorescent, but redder in shade than untreated wool.

When examining wool it should be remembered that the oils used in lubricating the yarn may modify the fluorescence, and that exposure to light for some weeks or to ultra-violet light for some hours, may cause a very marked dulling of the fluorescence. The detection of mechanical or chemical damage to wool is dealt with on page 368.

Mohair appears bluish-white and cannot be distinguished from wool, white calf or cow hair appear redder in tone, and white pig bristles show a bluish-white fluorescence.

Raw silks (*e.g.*, yellow Italian grege) have a yellow fluorescence, and there is some evidence for the opinion that the colour of the fluorescence under the lamp bears some relation to the colour of the raw silk in daylight. Even after degumming some silks show a yellowish colour, and have a slight dull yellowish fluorescence under the lamp. In one laboratory it was found that all the breaks that occurred in degummed silks took place in these yellow portions, and that they may be due to bacterial action on the bast.

According to H. Kaneko and others [19, 20] on drying *silk cocoons* the sericin undergoes hydration and alters in physical properties (*e.g.*, surface tension, viscosity, refractive index, turbidity and colloidal properties). When these are changed so as to produce better reeling properties, a yellow fluorescence is developed (*cf.* p. 247). According to Frehse,[21] certain silks do not fluoresce, although they contain loadings, the ashes of which appear faint blue. This worker also mentions the different fluorescence colours of silks dyed to the same tint and apparently the same when viewed in daylight (see also Desmurs [40] and p. 340).

M. Oku [25] finds that coloured silk cocoons, in general, have a yellow fluorescence, but that when the colouring matter of the cocoon is extracted by solvents, the cocoon has a violet fluorescence, which may be due to sericin and fibroin. Of the pigments which can be isolated from the various types of cocoons, lutein, violaxanthin, carotene, bombycin and bombycetin all have a yellow fluorescence (see also p. 264).

Mixed Fabrics.—Cotton and linen can be distinguished from half-linen, especially if the edges of the fabric are frayed and examined under the lamp. Raw half-wool fabrics often allow the cotton to be detected by the less intense fluorescence; these threads show in contrast to the wool, *e.g.*, wool-Fibro does not appear so bright as wool delaine, but it has the same shade of fluorescence colour and tiny dark spots show where the cotton fibres are exposed. By means of the above reagents used in conjunction with fluorescence microscopy, mixtures of viscose, Bemberg and cellulose acetate rayons with one another can be differentiated (*cf.* Radley, p. 368).

It is interesting to note that the property of *selective adsorption* by certain fibres for fluorescent compounds is being used to obtain a differentiation of these fibres (see J. Grant [50]). A. Segitz [22] suggests such a method for Cellon and Cellophane, and utilises the different adsorptions of the two substances for the violet fluorescent compound contained in spruce bark extract. This compound is adsorbed by Cellophane but not by Cellon, so that after treatment the former appears violet under the lamp whilst the latter remains colourless. This method has also been used [38] for the separation of cotton or string from wool on a commercial scale, the mixed material being treated with Primuline-*AS* which stains the string thus allowing it to be separated more readily.

Examination in ultra-violet light has been used [56] to supplement determinations of the urea and starch-contents in the evaluation of bedding and upholstery materials. The difference in fluorescence which exists between new and old kapoks enables new kapok and admixtures of new and second-hand materials to be detected.

Rayons.—Le Trayas [11] gives a table showing the fluorescence of a number of direct dyes on rayons and natural silks, and mentions that, with the same dyestuffs, cotton and rayons of the regenerated cellulose type have similar fluorescence colours, whilst natural silk and viscose show pronounced differences in shades.

W. Weltzien,[12] R. Cunz and R. Lassé,[13] E. Göthel,[31] M. Nopitsch [14, 15] and P. Picavet [16] have examined a large number of fibres from various sources which had undergone different manufacturing treatments. Picavet considers that differentiation by fluorescence is not sufficiently safe as a means of classification of

rayons, and it may be noted that rayons of the same type often differ in fluorescence owing to variations in the method of treating the fibre before it is marketed; this may explain apparent contradictions in the work of some investigators. Picavet's results are included in Table 31 :—

TABLE 31.

Cuprotextile (cuprammonium silk).	White.
Cuprotextile (made from linters by stretch-spinning).	Blue-white.[30]
Bemberg (cuprammonium silk).	White
Celta silk (viscose).	Yellow.
Aubenton silk (viscose).	Yellow.
Snia Viscosa silk (viscose).	Dull violet.
Cetilose silk (viscose).	Brilliant white.
Chardonnet silk (nitrate silk).	Pure yellow.
Brysilka (bleached and sized, from sulphite pulp).	Yellow.[30]
Japp silk (natural silk).	Dark yellow-brown
Casein "wool"	Dead white.[37]

The differences in fluorescence are sufficient to distinguish Nos. 1 and 3 from 4 and 5, whilst the colour of the fluorescence of No. 10 is strongly characteristic. Mixtures of the rayons and cotton in any proportions show no differences in fluorescence under the lamp. Cellulose acetate products are in general brighter than the others and show tones of blue-violet to violet, but the sizing affects the intensity of the fluorescence.[30] J. M. Preston [32] has published some interesting photographs in this connection, and was able to demonstrate the rayon effect in corset threads which are invisible in ordinary light (Photograph No. 22, facing p. 400). H. Yorke [33] reviews the chemical, microscopical and ultra-violet tests for rayons (see also J. A. Radley [26]), and Munzinger [45] has demonstrated the influence on the fluorescence of the plasticiser used.

The fluorescence of the rayon silks is unaffected by extraction with light-petroleum, but after treatment with 96 per cent. alcohol, the fluorescence of *cellulose acetate* becomes less intense and yellow-grey in colour. *Cuprammonium* and *viscose silks*, when scoured with a solution containing 0·5 per cent. of soap and 0·5 per cent. of ammonia, show no change in fluorescence, but the fluorescence of cellulose acetate silk, on the other hand, is paler and less blue. When viscose silk is treated with caustic soda of sp. gr. 1·008, no change in the fluorescence is observed,

but with cuprammonium silk the colour changes to grey and then to reddish-violet, whilst that of cellulose acetate changes to pale yellow. Fluorescence microscopy may be used to identify rayons, *e.g.*, M. Haitinger [44] was able to distinguish viscose (yellow-green) from acetate (blue) by means of thio-flavin-S (see also p. 366).

Bemberg rayon can be distinguished from viscose rayon by the following method, due to J. A. Radley.[39] A cold solution of 0·2 grm. of Acronol Yellow-*TS* is made in 100 c.c. of a mixture of equal parts of water and methylated spirits, to which 5 c.c. of a 10 per cent. sodium carbonate solution have been added, the solution being boiled and cooled. The sample to be examined is immersed for 2 min. in the cold solution, and is then thoroughly rinsed in cold water and examined under the lamp. The viscose appears a dull purple or dull blue, whereas Bemberg silk appears a brilliant blue or yellowish-white according to the state of dryness of the sample and the duration of the treatment.

If cellulose acetate is dipped for a second or two in the above reagent, and rinsed and examined, it shows a vivid deep blue colour which is most striking, and which easily distinguishes it from the colours shown by either viscose or Bemberg rayons. In these tests it will be noted that the fabrics are examined in the wet state. The intensity of the fluorescence is somewhat diminished by drying, but the colours are rather more distinctive.

Another test for distinguishing between Bemberg and viscose materials (see J. A. Radley [46]) is carried out by "dyeing" the sample at 85° C. for 5 min. with Primuline-*AS*, using a 1 per cent. shade, and a 30 : 1 liquor. Prior to entering the sample in the dyebath, concentrated sulphuric acid to the extent of 5 per cent. on the weight of the hank is added to the liquor. After rinsing the viscose has a bright blue fluorescence, whereas the fluorescence of the Bemberg material is golden yellow.

The Detection of Faults.—Faults may be present in the raw materials used in a process or they may be introduced by the process itself. Even after the article is finished it may become damaged by light, water, gas fumes, or contamination.[39] W. Sieber [3] gives a method whereby mechanical or chemical damage to wool can be detected. After immersion in a boiling 1 per cent. aqueous solution of benzo-purpurin-10*B*, followed by washing, undamaged wool remains colourless, mechanically-damaged

wool becomes pink, and wool attacked by alkali or acid becomes yellowish-pink to red or bluish-pink to red, respectively. These colour changes are much more apparent under the lamp. Ordinarily, over-carbonated wool, or over-bleached or acid-damaged cotton or flax shows no particular change, whilst alkali-damaged wool appears somewhat duller.

In dyeing or bleaching the detection of the presence of mineral oil in the materials is of great importance. Fortunately, under the lamp, *mineral oil*, even in very small traces, is clearly seen by its bright bluish-white fluorescence (see Photographs Nos. 7-9, facing p. 400), but it is interesting to note that after some hours of exposure to sunlight the colour of this fluorescence changes to a yellowish-white similar to that given by plant oils. G. Blaser and Girsberger [6] have worked out a method for the determination of the amount of mineral oil on a fabric.

Irregular dyeing on wool may result from stains due to mineral or fatty oils ; Hirst [29, 55] was one of the first workers on this subject. The former appear blue in ultra-violet light, although after 60 hours of exposure they assume a white tinge (due to the formation of a yellow precipitate which is accompanied by an increase in the viscosity of the oil) ; the latter (*e.g.*, oleic acid or cotton-seed oil) turn from blue to yellow after 12 days under the carbon arc, and the colour eventually disappears after 72 days. After exposure the two types of oil attract and resist acid dyes on wool, respectively. Staining oils have a brilliant surface glow, whilst others may show fluorescence to a considerable depth. Pure, saturated hydrocarbons are distinguished (on cloth) from unsaturated hydrocarbons and resinous stains by the brighter fluorescence of the two last-named, the colours of which are usually blue and green respectively. There appears to be some connection between the fluorescence and the degree of exposure to the atmosphere of oils spotted on cloth. As C. A. Amick [37] points out, the presence of dirty grease may mask the fluorescence of oil stains, and this may be overcome by addition of a drop of ether while the sample is under the lamp, when a fluorescent ring is formed.

For the *standardisation of oils* H. R. Hirst [29] compares the fluorescence with that of varying mixtures of quinine sulphate (blue) and Indigosol-O (violet), the sample being diluted with petroleum spirit if necessary.

It should be pointed out that such oil stains are not plainly visible under the lamp if the cloth is heavily dyed, but if the colour is stripped using as gentle a process as possible having regard to the particular dyestuff employed, the oil stain will fluoresce brightly (see J. A. Radley [47]).

Single strands, showing a bright fluorescence, sometimes indicate that the thread has been contaminated in the weaving but patches of bright blue in which all the threads fluoresce, indicate that the cloth has been contaminated with oil spots after weaving.

In bulk, distilled olein gives a clear violet fluorescence, but if spotted on the cloth some samples show a fairly bright white fluorescence. Saponified olein in bulk gives a buff fluorescence, the browning of the colour probably being due to an increase in the proportion of oxidised fatty acids. Samples of oleic acid can vary greatly in their colour under the lamp, some pure samples showing no fluorescence, whilst redistilled samples appear violet. One of the chief uses of the method here is to check deliveries of oil against the sample of an oil ordered. Fatty oils from vegetable sources are sometimes contaminated with chlorophyll which gives a red fluorescence (see p. 299), which may become predominant in oils containing much of this compound. If present in oleic acid it can mask the blue fluorescence of the latter. In actual practice, the oils used are generally mixed fatty acids or glycerides, so that it is preferable to record descriptions of the appearance of oils of known origin when examined in ultra-violet light. Damage by bleach spots on cotton piece is readily detectable by the variation in fluorescence at such points [55] (see also Chapter XX).

In a recent printing process,[52] highly-fluorescent inert substances are added to the print pastes, and an ultra-violet lamp is fixed in front of the printed fabric. It is claimed that scumming and snaps, etc., can readily be detected in this manner, and that this process is especially applicable to dyestuffs which give colourless printing pastes such as certain Indigosols and Soledons, Rapid Fast Colours and discharges.

Light damaged materials yield interesting results. Wool stuffs are dull and grey, and degummed silk no longer fluoresces white but appears yellow in colour and similar to raw silk. Plant fibres gradually assume a dark violet fluorescence which deepens

on continued exposure to light. Certain blemishes in materials due to oil spots, moulds and metal spots are more easily detected under the lamp than in daylight (see Photographs Nos. 7-9, 16, 18 and 22 facing p. 400.). To differentiate between textiles such as cotton or linen which have been tendered by chemic and by acid, the samples are baked on a white tile for 45 mins. at 140° to 155° C. The former turn brown and have a greenish-blue fluorescence, whilst acid-tendered samples show little colour change in colour in daylight and have no fluorescence. H. Haas [58] describes changes in the fluorescence of raw cotton which occur as the result of heat treatment.

Another observation which, however, has no practical importance, is that the fading of fluorescent dyestuffs and light damage of certain fibres can be followed by the decrease in their fluorescence under lamp ; such a decrease sometimes occurs before it is apparent visually and generally it proceeds much more rapidly than the fading. This takes place with fabrics, lacquers and films, and in the last two cases there may occur a change in shade in reflected visible light owing to the loss of the slight fluorescence which is visible in daylight ; however, there is not then necessarily any difference between the appearance of the exposed and unexposed portions when viewed by transmitted visible light.

If degummed silk damaged by light is examined under the lamp it no longer appears bluish or white but exhibits the yellow tone shown by the raw silk, this change according to A. Castiglioni[48] being due to the action of sulphur acids on traces of cholesterol present.

The " marking-off " of dyestuffs on acetate silk can often be detected under the lamp, but in most cases this is apparent to the unaided eye, so that no advantage is obtained. In the case of some vat colours, which tend to " mark off " in laundry work, minute amounts invisible to the eye in daylight can, however, readily be detected under the lamp.

Stains.—Oil stains have already been mentioned above. Paint stains may show practically no fluorescence, or may appear a dirty purple brown. The application of a few drops of solvent to such spots will produce a bright fluorescent ring, often yellow or greenish-yellow in colour, while the pigment which is usually non-fluorescent, is left in the centre. Dirty varnish stains behave similarly, and give greenish-yellow rings on spotting with

solvents, and very brilliant stains are obtained in this way fron tar or bitumen. Tea stains do not fluoresce, but coffee stain show a brilliant blue which is greatly increased in intensity i the coffee has been sweetened with sugar. Chlorophyll stain have a blood-red fluorescence in the presence of solvents fo chlorophyll (see p. 299). Ink stains, especially if made on a coloured material, normally appear dark, but red ink based or Eosin Y 125 is highly fluorescent. The colour of the materia investigated will, naturally, affect the results just as indicated previously in connection with mineral oil stains.

Wetting agents based on naphthalene sulphonic acids or their salts, and sulphoricinoleates, have a strong fluorescence in the mass. A stain made by a 1 per cent. solution of a wetting agent of one of the above types on a white material shows a blue fluorescence under the lamp.[14]

Nopitsch[14] states that even after boiling and bleaching, traces of these wetting agents may still frequently be seen. The naphthalene sulphonic acids and their salts have violet to blue-rose fluorescence colours which are more intense than those of the sulphoricinoleates and their salts, and when spotted on to fabrics they generally show a brighter outer edge or halo which is absent from spots made by sulphoricinoleates. The latter, which fluoresce in yellow-white to blue-white shades when spotted on fabric do, however, show a halo sometimes if used with a fat solvent.

Sizing and Dressing Agents.—For the identification of different substances, *e.g.*, used in sizing and dressing agents the lamp has been found to be of value. Starches and dextrins are fluorescent in the massive state, but the colour of the fluorescence does not assist the identification of the starch or dextrin used in textile sizes.

Waxes have a dull fluorescence in the massive state but on the fibre the only two that can be detected when present in reasonably small quantities are paraffin and carnauba, more particularly the latter (see p. 307). A number of metallic compounds present in a dressing may be detected by means of a reaction which gives a fluorescent end-product, and this is a method of general application. Thus a 10 grm. portion of cloth may be extracted with 100 c.c. of boiling water for 10 minutes, and after decantation the liquid retained in the cloth is squeezed out into the main bulk of

liquid which is then evaporated to about 25 c.c. and any soluble starch or dextrin precipitated by the addition of 3 to 4 times the volume of 96 per cent. alcohol; the mixture is then filtered. Borates or boric acid may be detected by acidifying a portion of this extract with acetic acid, boiling, and adding a few c.c. of the liquor to a few c.c. of a solution of cochineal, buffered to *p*H 5·8 to 6·9 with a phosphate buffer. The presence of boric acid is then shown by a brilliant yellowish-orange glow under the lamp; or by adding a 0·5 per cent. solution of Alizarin Red *AS* to the neutralised solution, followed by an equal volume of alcohol, when a yellowish-red fluorescence is obtained.

Zinc salts may be detected by making a portion of the extract slightly alkaline with sodium hydroxide solution, and then acid again with acetic acid, and adding a few drops of this liquid to an alcoholic solution of *o*-hydroxyquinoline, when a brilliant yellowish-green fluorescence appears if zinc is present. Magnesium also gives a golden-yellow fluorescence in this test, but a specific test for zinc is obtained by using a solution of urobilin instead of *o*-hydroxyquinoline, when a characteristic brilliant green fluorescence is obtained (see p. 202).

Aluminium salts are detected by adding a few drops of the extract to an alcoholic solution of tetrahydroxyflavonol (morin or old fustic), when a bright greenish-yellow fluorescence is shown in the presence of minute amounts of aluminium (see p. 205). If a piece of cloth is spotted with a solution in alcohol of the above reagent and examined both before and after the spots have dried, the characteristic fluorescence colours can be seen quite distinctly if the metals concerned are present.

J. A. Radley has applied this method to the examination of effects of storage on grey cloth. Zinc chloride is used in warp-sizing and is at first entirely on the warp; if, however, it is stored under damp conditions, it is frequently found on both warp and weft. The urobilin test must be used as grey cloth often contains appreciable amounts of magnesium salts, and an alcohol solution of urobilin is therefore sprayed with a scent spray on to the cloth to be tested for 1 to 2 seconds and the sample is examined under the lamp. With a freshly-processed cloth which has been stored in a dry place the warp threads only will be seen to be bright green, but with mildewed cloths or those stored in a very damp atmosphere, both warp and weft will be green.

J. A. Radley[46] has devised a test to detect or confirm aluminium salts in presence of magnesium and zinc salts, and of other salts (*e.g.*, calcium salts) or compounds commonly occurring in textile sizes and dressings. The test may be carried out by gently ashing the sized fabric or the size itself, dissolving the ash in dilute hydrochloric acid, and almost neutralising the solution with caustic sodium hydroxide solution. One c.c. of a 0·2 per cent. solution of Solochrome Violet *RS* is then added, and the liquid is boiled, cooled, and diluted with an equal volume of 95 per cent. alcohol and examined under the lamp, when a brilliant orange fluorescence is obtained in the presence of 1 part per million of aluminium in the solution. Zinc salts give a dull blood-red fluorescence, but this does not interfere with the test even when they are present in a great excess, as the orange fluorescence given by aluminium is so brilliant and distinctive. Solochrome Red *ERS* or Solochrome Dark Blue *BS* may also be used.

If desired a dressed fabric or a size may be extracted with hot water, without ashing, and the test carried out on the filtered solution. In many cases a solution of the dyestuff in aqueous alcohol applied directly to the fabric and examined while wet gives the brilliant orange fluorescence, and it is rare indeed that this latter method fails to detect aluminium if this is present in any appreciable amount.

The lamp gives very little information of analytical value regarding the weighting agents present, as the fluorescence of pigment whites so often depends on the method of manufacture and the impurities present (see J. Grant, p. 340). Talc in a dressing, however, can be detected by ashing a 2 grm. portion of the dressing or finished cloth, extracting the ash with hot water, treating the residue with dilute hydrochloric acid, washing with hot water to remove the soluble salts, and evaporating in a platinum dish with concentrated hydrochloric acid. The residue is dissolved in a little water and tested with the alcoholic solution of *o*-hydroxyquinoline sulphate to show the presence of magnesium; this reaction is given by talc but not by kaolin. The alcohol solution of morin used to detect the presence of aluminium or, preferably, Solochrome Violet *RS* solution, will also indicate the presence of kaolin or china clay.

Urea formaldehyde resins fluoresce, but phenol formalde-

hyde resins do not.[53] Phthalic anhydride resins may be extracted with a solvent, which is then evaporated and the residue treated on the water-bath with a few crystals of resorcinol and a few drops of concentrated sulphuric acid, or heated to 130° C. for 5 minutes. On diluting the mass and adding sodium hydroxide solution, an intense fluorescence is obtained from resins of this type.

Casein has a strong bluish-white fluorescence, and it should be possible to apply the Voges-Proskauer reaction which was used by A. Harden and D. Norris [54] to detect protein material (see p. 308). Gum arabic has a bluish fluorescence which is not nearly so strong as that of albumen, whilst gelatine also gives a bluish-white fluorescence. Gum tragacanth on textiles is not easily detected, as it has only a weak whitish fluorescence which cannot easily be seen.

In view of the fact that these sizes appear very similar on textiles and that various oils and fats may be included in the sizing mix, little information can be gained as to the presence or otherwise of gums, starches or dextrins by direct observation under the lamp. Other work on the subject will, however, be found in the references.[42-50]

REFERENCES.

[1] H. Sommer, *Textilber.*, 1928, 9, 753.
[2] H. Sommer, *Woch. Papierfabr.*, 1928, 60, 9.
[3] W. Sieber, *Textilber.*, 1928, 9, 326.
[4] S. Moskowitz, W. Landes and D. Himmelfarb, *Amer. Dyestuff Reporter*, 1936, 25, 220.
[5] A. W. Winne and J. D. Donovan, *ibid.*, 1932, 21, 601; Priv. Comm., 1939, Feb.
[6] G. Blaser and Girsberger, Private Communication; *Mell. Text.*, 1929, 10, 963.
[7] S. Moskowitz, W. Landes and D. Himmelfarb, *Amer. Dyestuff Reporter*, 1935, 24, 463.
[8] E. Gründsteidl, *Chem. Zentr.*, 1932, ii, 2261.
[9] E. Gründsteidl and F. Hanika, *Ind. Text. Jour.*, 1935, 45, 172.
[10] O. Mecheels and E. Gründsteidl, *Text. Manuf.*, 1934, 60, 434.
[11] Le Trayas, *Russa*, 1929, 2, 171; *Art. Silk World*, 1929, 460.
[12] W. Weltzien, *Seide*, 1928, 33, 306.
[13] R. Cunz and R. Lassé, *Textilber.*, 1928, 9, No. 12.
[14] M. Nopitsch, *ibid.*, 1928, 9, 136, 241, 330.
[15] M. Nopitsch, *Kunstseide*, 1928, 10, 321.
[16] P. Picavet, *Russa*, 1929, 4, 539.
[17] W. H. Ellinger and L. Windschauer, *Textilber.*, 1930, 11, 61.

[18] W. H. Ellinger, *Jentgen's Rayon Rev.*, 1930, **2**, 96.

[19] H. Kaneko, T. Hayashi, S. Chino and M. Miyasaka, *Bull. Sericult. Silk Ind. Japan*, 1930, **2**, 2 (*Brit. Chem. Abst. B.*, 1930, 857).

[20] H. Kaneko and M. Miyasaka, *ibid.*, 1931, **3**, 1 (*Brit. Chem. Abst. B.*, 1931, 532).

[21] Frehse, *Ann. Falsif.*, 1925, **18**, 204.

[22] A. Segitz, *Papier Fabrik.*, 1930, **28**, 206.

[23] L. Meunier and G. Rey, *Compt. rend.*, 1926, **183**, 596.

[24] W. Weltzien, *Seide*, 1928, **33**, 306; 1930, **35**, 195.

[25] M. Oku, *Jour. Agric. Chem. Soc. Japan*, 1934, **10**, 1239, 1253.

[26] J. A. Radley, *Rayon Record*, 1933, **7**, 21.

[27] E. Grünsteidl and F. Hanika, *Mell. Text.*, 1934, **15**, 88, 93; *Faserforsch.*, 1933, **10**, 215; *Zeit. Ges. Text. Ind.*, 1932, **35**, No. 32.

[28] "Standards for Light, Perspiration and Washing" (Bradford: Society of Dyers and Colourists, 1934).

[29] H. R. Hirst, *Jour. Soc. Dyers and Col.*, 1931, **47**, 347; 1932, **48**, 90; 1934, **50**, 211; *Amer. Dyestuff Reporter*, 1931, **20**, 347; 1932, **21**, 90; 1934, **23**, 559.

[30] R. H. Marriott, "Allen's Commercial Organic Analysis," 5th edn., vol. x. (London: J. & A. Churchill, 1933).

[31] E. Göthel, *Diss.*, Dresden, 1933; *Mell. Text.*, 1934, **15**, 409.

[32] J. M. Preston, "Modern Textile Microscopy" (Manchester: Emmott, 1933).

[33] H. Yorke, *Dyer*, 1933, **69**, 307.

[34] G. Desmurs, *Rév. Gén. Teint. Imp. Blanch.*, 1932, **10**, 231, 307, 417, 505.

[35] J. L. McEwen, *Amer. Dyestuff Reporter*, 1932, **21**, 337.

[36] R. Lassé, *Mell. Text.*, 1931, **12**, 249.

[37] Q. Hirsch, *Rév. Gén. Teint. Imp. Blanch.*, 1926, **6**, 541; *Dyer Cal. Printer*, 1930, **63**, 393.

[38] String Detector Co., U.S.P. 2,046,940, 1936.

[39] J. A. Radley, *Jour. Soc. Dyers Col.*, 1937, **53**, 376.

[40] G. Desmurs, *Tiba*, 1932, 10,231.

[41] T. H. Daugherty and E. V. Hjort, *Jour. Ind. Eng. Chem. Anal.*, 1934, **6**, 370.

[42] A. E. Sampson, *Amer. Dyestuff Reporter*, 1935, **24**, 8.

[43] Sub-Committee on Light Fastness, *ibid.*, 1934, **23**, 619.

[44] M. Haitinger, *Mikrochemie*, 1935, **16**, 321.

[45] W. M. Munzinger, *Kunststoffe*, Dec., 1934.

[46] J. A. Radley, *Analyst*, 1938, **63**, 266.

[47] Lunge-Keane, "Technical Methods of Chemical Analysis."

[48] A. Castiglioni, *Chim. e Ind.*, 1935, **17**, 82.

[49] M. Déribéré, "Applications de la Lumière de Wood et des Rayons Ultra Violets dans les Industries Textile et Tinctoriale" (Paris: Editions Textile et Technique), 1937.

[50] J. Grant, *Jour. Soc. Dyers Col.*, 1938, **54**, 361.

[51] J. Grant, *Textile Col.*, 1936, **58**, 153.

[52] Anon, *Textilber., Eng. Ed.*, 1937, **28**, 117.

[53] G. Kostka, *Chem. Ztg.*, 1929, **117**, 138.

[54] A. Harden and D. Norris, *J. Physiol.*, 1911, **42**, 332.

[55] H. R. Hirst, *Jour. Soc. Dyers and Col.*, 1937, **53**, 45.

[56] Anon, *Analyst*, 1938, **63**, 894.

[57] C. M. Whittaker, *Jour. Soc. Dyers and Col.*, 1937, **53**, 468.

[58] H. Haas, *Deut. Woll. Gewerbe*, 1937, **69**, 1541.

ADDITIONAL REFERENCES.

F. T. Addyman, *Dyer and Calico Printer*, 1929, 61, 184, 227, 343.

H. Stadlinger, *Textilber.*, 1930, 11, 450 (Testing rayons).

J. Muir, *Ind. Chem.*, 1932, 8, 348 (Testing sizes, detecting faults and examination of dyestuffs).

Anon, *Bull. Shirley Inst.*, 1932, 5, 218 (Detection of stains on textiles).

K. Trager, *Kunstseide*, 1935, 17, 139 (Differentiation of fibres).

Anon, *Silk and Rayon*, 1935, 9, 593 (Titration of dyestuff solutions in ultra-violet light).

R. Mitcheson, *Dyer*, 1938, 79, 41 (Differentiation of rayons).

Anon, *Ciba Rundschau*, 1938, 1079 (General).

CHAPTER XIX.

WATERS AND SEWAGE.

A NUMBER of workers have recorded the various effects noted when waters are examined under the lamp, but these observations are of little use in routine work.

Water.—*Pure distilled water* is practically devoid of fluorescence ; the faint blue colour observed has been attributed by S. J. Vavilov and L. A. Tummermann [6] to oxygen or carbon dioxide, since it is also shown by many liquids with similar spectral features (*e.g.*, sulphuric acid, ether, acetone and benzene). Ordinary *tap water* has generally a faint bluish fluorescence, due, in all probability, to small traces of dissolved organic matter. H. Ivekovic [1] has compared the fluorescence of drinking waters with gelatine standards, and claims to have found that the intensity of the bluish-white fluorescence is proportional to the oxygen absorption as measured by the potassium permanganate demand. He also attributes the fluorescence to organic matter, but as L. Francesconi and R. Bruna [11] have shown, certain radioactive metals produce a green colour.

J. Duclaux and P. Jeantet [2] mention that *polluted water*, and water containing proteins and ammonia, can be readily distinguished from pure water. They deal chiefly with the transparency of water towards ultra-violet light, and note that *rain water* is inexplicably opaque ; this may be due to its freedom from suspended impurities. Mineral salts appear to have no influence on the degree of transparency, and it is not possible to distinguish between waters containing nitrites and those containing nitrates by means of the ultra-violet spectrum.

Sewage.—J. A. Radley [3] has examined effluents running into the river from a sewage works in which the sewage was partly treated by the activated-sludge process, and then subjected to land treatment. A bluish-white fluorescence was nearly always

noted, although sometimes the colour was greenish-white. The intensity, which was greatest in alkaline solution and often quite strong in neutral solutions, appeared to have no connection with the potassium permanganate demand (4 hours) or with the free- or albuminoid-nitrogen figures, even after filtration. The fluorescence disappeared when the water was made acid, and returned when it was made alkaline.

The titration of sewages with fluorescein as a fluorescent indicator (*cf.* p. 311) has no advantage over the usual methods of titration to methyl orange. A strongly-fluorescent substance can be extracted from raw sewage in ether, but not in carbon tetrachloride, and it does not pass into the distillate on steam-distillation. Evaporation of the ether-extract yields a scum which fluoresces with an intense reddish-yellow colour, and a watery liquid having an intense reddish-blue fluorescence. The scum is fatty in nature and only partly saponifiable, and the residue after evaporation of the liquid gave Reif's fluorescence test for creatinine (see p. 308).

Fresh crude *sludges* appear very dark under the lamp and show a faint greenish-grey fluorescence with a number of intensely fluorescent specks, blue, green and yellow in colour. These specks, which appear to be due to particles of fats and cellulose, gradually disappear as the digestion of the sludge proceeds, and are practically absent from a fully-digested sludge. The capillary method of Danckwortt and Pfau, however (p. 58), gave negative results. The *mucus* on the filter-bed media gave a red or orange fluorescence with patches of bluish-grey.

E. Chemin and J. Turchini,[4] who examined *algæ* under the lamp, found that no fluorescence was produced with the green and brown varieties. On the other hand, red algæ gave a brick-red fluorescence, the intensity of which was dependent on the natural colour of the intact algæ. This fluorescence is attributed to the phycoerythrin, which is originally confined to the plastids but passes into the cell-cavity, when algæ begin to spoil, with the production of an orange fluorescence. The fluorescence of certain bacteria found in waters is dealt with on page 98. Water from tanks containing marine organisms or plants contains a fluorescent material which passes through filter-candles. According to E. Merker [7] (*cf.* p. 100) it is a renal secretion, although the origin in the case of plants is unknown. The work of Dhéré

and others[8-10] on the pigments of water growths and organisms is discussed on p. 253.

Little other work appears to have been done on waters, but a number of workers mention the Tyndall effect in ultra-violet light, and state that on purification of the water the effect alters until it becomes constant for pure water. *Water vapour* at 0° C. and 4·6 mm. pressure illuminated with light of wave-length 2537 Å. emits, according to F. Rasetti,[5] an intense ultra-violet light.

REFERENCES.

[1] H. Ivekovic, *Zeit. Hyg. Infectionskrankh.*, 1931, **112**, 54.
[2] J. Duclaux and P. Jeantet, *Compt. rend.*, 1925, **181**, 630.
[3] J. A. Radley, *Analyst*, 1932, **57**, 28.
[4] E. Chemin and J. Turchini, *Bull. Inst. Oceanogr.* (*Monaco*), 1930, 1.
[5] F. Rasetti, *N. Cimento*, 1931, **8**, 191.
[6] S. J. Vavilov and L. A. Tummermann, *Zeit. Phys.*, 1929, **54**, 270.
[7] E. Merker, *Naturwiss.*, 1931, **19**, 433; *Zoöl. Jahrb. Physiol.*, 1931, **49**, 69.
[8] C. Dhéré and M. Fontaine, *Compt. rend.*, 1931, **192**, 131; *Ann. Inst. Oceanogr.*, 1931, **10**, 249 (Algoid pigments).
[9] E. Bachrach and C. Dhéré, *Compt. rend. Soc. Biol.*, 1931, **108**, 385 (Diatoms and the chlorophyll pigments).
[10] C. Dhéré and M. Fontaine, *ibid.*, 1931, **105**, 843 (Bonellins).
[11] L. Francesconi and R. Bruna, *Gazz.*, 1934, **64**, 485.

CHAPTER XX.

THE DYESTUFFS INDUSTRIES.

A LARGE amount of work [86] has been carried out using the lamp for the examination of dyestuffs, much of it directed towards fundamental research into the laws governing fluorescence phenomena. As certain dyestuffs have an intense fluorescence under widely varying conditions they are ideal subjects for investigations on the influence of temperature, solvents, etc., on the fluorescence. A study of these phenomena is outside the scope of this volume, but a short discussion of them is desirable if only to illustrate the nature of the conditions which influence fluorescence effects.

The use of the lamp in connection with dyestuffs may be conveniently classified under the following main headings :—

1. Fundamental research into the laws of fluorescence.
2. Classification, and in a few instances, identification of dyestuffs.
3. Testing the resistance of dyestuffs to the action of light (accelerated fading).
4. The detection of faults in dyeing and printing.

1. Fundamental Research.

Influence of Solvents and Concentration.—The nature of the fluorescence of any dyestuff, or indeed of any fluorescent compound, depends upon the type of solvent in which it is dissolved, and the intensity varies with the concentration. The fluorescence is sometimes perceptible even in dilutions so great as 1 in $10,^7$ but dilutions of 1 in 20,000 or 1 in 200,000 usually give the best results for the examination of dyestuffs in the dyeing or textile laboratory (see p. 388).

The effect of concentration and of viscosity has been studied by A. V. Banov [60] who found that, for a number of dyes, a 300-fold

increase in viscosity has no effect on the fluorescence. A change in the fluorescence-spectra of Eosin-G or fluorescein in glycerol, Erythrosin-B in ethyl alcohol, and Rhodamine-B in methyl alcohol solution occurs at high concentrations, and this change is shown by those dyes in which the diminution in fluorescence is slow; where this diminution is rapid the fluorescence spectra are unchanged. The nature of the solvent also determines the extent of the variation of fluorescence with concentration. J. Bouchard [61] found that the logarithm of the intensity varies linearly with the concentration independently of the solvent (*cf.* Perrin's law [62]), and is a function of the molecular velocity of diffusion. Differences occur even with dyes examined in the solid state, *e.g.*, auramine obtained in a finely-divided form by evaporation contains glowing particles (*cf.* P. Balavoine [63] and also M. Haitinger [64]).

V. L. Levschin,[1] using Rhodamine G extra (C.I. No. 750), finds that the absorption spectrum decreases in intensity with increasing concentration of dye owing to the association of the dyestuff molecules, these associated molecules being incapable of fluorescing. On heating the concentrated solution the associated molecules are reduced in number, and the solution again behaves like a dilute solution. G. Braun [2] concludes that when a dyestuff is dissolved in a mixture of solvents, two forms of the dyestuff molecule may be present, and these may well be the associated and unassociated forms. The fluorescence of a number of dyestuffs in glycerol has been examined by A. Wrzesińska [3, 4] and in a variety of solvents by J. Bouchard.[5]

Rhodamine-6G and eosin give a similar red-orange fluorescence in water, and green and no fluorescence, respectively, in concentrated sulphuric acid. On dilution the fluorescence increases to an optimum and then decreases, the extreme limits of visibility being from about 1 in 100 to 1 in 10^{-6} parts. In concentrated solutions it is only the surface of the liquid which fluoresces, because the radiations cannot penetrate below it. The fluorescence is often destroyed by the addition of salts, but when this is the case it is often regenerated by dilution. The dielectric constant, *p*H value, and the viscosity of non-aqueous solutions play a very important part in determining the colour and intensity of the fluorescence.

J. Bouchard [5] studied the effect of the dielectric constant (see p. 289) of the solvent on the intensity of the fluorescence, and

found that there is a linear relationship between the dielectric constant and the optimum concentration for the fluorescence.

According to C. Stora [6] the nature of the solvent also plays an important part on the fluorescent and photochemical behaviour of a dyestuff when irradiated.

Influence of Temperature.—The effects of concentration and changes in temperature on the fluorescence spectra have also been studied by F. G. Wick and C. G. Throop [65] for solutions of some of the above dyes, and by J. R. Jenness [66] for tetrachlorofluorescein and sulphonefluorescein in alcohol, and W. König and W. Regner [67] have studied the pentamethine series.

At very low temperatures, such as 202° A., fluorescein and gelatine become phosphorescent, the glow lasting, according to K. Ochai,[68] for about 30 seconds.

P. Fröhlich [7] has determined the temperature at which the maximum phosphorescence was obtained from solutions in alcohol or glycerol solutions of Eosin *A*, Rhodamine *S* (C.I. No. 743) and Erythrosin (C.I. Nos. 772 and 773). He found that there was a steady transition between fluorescence and phosphorescence, and no sharp change from one to the other. F. G. Wick and C. G. Throop [8] have cooled solutions of fluorescent dyestuffs to the temperature of liquid air, and find that marked changes in the colour and intensity of the fluorescence occur, and that in some cases the solutions became very strongly phosphorescent. The effect of heating concentrated solutions has already been dealt with above.

The Rate of Decay of Fluorescence.—The rate of decay of fluorescence when excitation is discontinued (*cf.* phosphorescence, p. 4) has been studied by W. Szymanowski,[9-11] who introduced an improved form of fluorometer for which a sensitiveness is claimed of 0·2 to 0·3 $\times$ 10^{-9} seconds. Using this instrument he has shown [10] that the decay of the fluorescence of Fluorescein (C.I. No. 766) in aqueous solution follows the exponential law. He considers that collisions of the second order cannot explain the extinction processes as revealed by the decay of fluorescence of Fluorescein in water, alcohol or glycerol. A. Jabłoński,[13] however, finds that the time decrement of polarised fluorescence does not always follow the exponential law, and Szymanowsky [11] considers that the rate of decay of the fluorescence excited by polarised light depends on the orientation of the

fluorescent radiation relative to the plane of polarisation. Another worker in this field, F. Duschinsky,[12] finds there is no difference between the degree of resistance to extinction of fluorescence and of phosphorescence.

I. C. Ghosh and S. B. Sen-Gupta [14, 42] find that the fluorescence effect is proportional to the wave-length of the exciting light, and appears to be independent of the concentration at high dilutions. On increasing the concentration beyond a certain point there is, probably, association of the dyestuff molecules, and this has been confirmed by the work of B. J. Sveschnikov [15, 16] (see also F. S. Barishanskaja,[17] Levschin,[18] Fonda,[19] and P. Pringsheim [20]), who followed the quenching by observing the increase of polarisation.

Inhibitors of Fluorescence.—Some inorganic compounds, such as the alkali halides, silver nitrate, potassium chromate and permanganate and the halogens, weaken or completely destroy the fluorescence, particularly in the case of fluorescein (F. M. Frank and S. J. Vavilov [69]), and the halogen derivatives of fluorescein show a diminished fluorescence, the extent of which varies according to the number of halogen atoms substituted and their nature. Similarly, the nitro-group depresses the fluorescence considerably, but the hydroxyl group often does so to a less extent. The alteration in the mean life of the fluorescence of eosin and the effect on it of varying quantities of potassium iodide have been measured by S. Boudin.[70]

The fluorescence of dyestuffs, like that of uranine [71, 72] and many other substances (see Chapters V and VIII, and p. 202) is extinguished by certain electrolytes, and A. V. Banov [73] found that in the case of eosin, fluorescein, erythrosin and the rhodamines, this corresponds approximately with the salting-out activity of the ions (*e.g.*, $Br' > Cl' > NO_3'$, and $Na^\cdot > K^\cdot > Li^\cdot > NH_4^\cdot$).

C. Achard and co-workers [21] have shown that the *geno*-alkaloids, *e.g.*, *geno*-strychnine hydrochloride, do not influence the fluorescence of aqueous solutions of Fluorescein (C.I. No. 766) in the same way as does the normal alkaloid, and this is attributed to the lack of anti-oxygenic effect of the *geno*-compounds. According to K. Weber [22] the power of extinction shown by the halogens ions in solutions of pH value over 7 depends on the normal potential of the ions. If this property is considered as a form of photo-chemical oxidation, then for strongly acidic

solutions a formula can be derived from the kinetic laws which agrees with the observed data. J. Bouchard,[23] using aqueous solutions of Fluorescein (C.I. No. 766), has studied the effect of adding potassium chloride and nitrate, and sodium acetate and sulphate, on the fluorescence. The decrease in the fluorescence thus brought about is attributed by this worker to the increased molecular association of the dyestuff.

H. Hellström [24] has studied the effect of ferrous ions on the fluorescence of Methylene Blue (C.I. No. 922), which is rapidly diminished as the amount of ferrous ion is increased, the extinction-velocity being dependent on the *p*H value of the solution. This worker considers that there may be an unstable compound formed between the Methylene Blue and the iron, which readily dissociates. K. Weber,[25] however, explains this in terms of the oxidation-reduction potentials, and points out that the iron-citrate complex used in Hellström's experiments (which were carried out in a citrate buffer solution), has a potential which decreases rapidly with an increase in *p*H value, and hence the energy required by the methylene blue reduction decreases. On the other hand, more energy should be required for the reduction at higher *p*H values owing to the $H^{\cdot}$ ion equilibrium. These two influences counteract each other so that the reduction rate passes through a minimum at *p*H 3. Weber considers that the phenomenon is related to the fading of vat dyestuffs in the presence of ferrous ions, so that both are dependent on the more positive redox potential of the activated dyestuff molecule or ion and its consequent reaction with the ferrous ions.

J. Weiss [43] has noted that many dyestuffs of the basic type which absorb in the visible region are reduced in the presence of inorganic reducing substances when irradiated. This photochemical reduction is dealt with in detail by this worker,[44] who finds that the leuco-base of the dyestuff is precipitated quantitatively from an aqueous solution in the absence of air when ferrous sulphate is present. If acid is present then the ferric ions formed on irradiation are not precipitated but remain in solution, and when the irradiation ceases they re-oxidise the leuco-body back to the dyestuff, so that the reaction is a rapidly-established photo-chemical, oxidation-reduction equilibrium. Similar results have been obtained by Euler and his co-workers,[45] and by K. Weber.[46] The reaction is not confined to ferrous salts,

the point of view of both the textile chemist and the dyer. The usual limits of visibility in this case are between 0·01 and 5 per cent. (weight of dye to weight of fibre). The effect varies in intensity according to the dye; thus Thioflavin-S gives a deep blue fluorescence on acetyl cellulose, but yellow on cotton or viscose, and green-yellow on silk or silk-wool mixtures.

The colour of the fluorescence depends on its intensity, since the fluorescent light undergoes selective absorption by the dyestuff itself. The effect of this absorption varies with the concentration, and is a confusing factor.

The majority of dyestuffs do not fluoresce in the solid state, and when materials are dyed with non-fluorescing dyestuffs the natural fluorescence shown by the substratum is repressed, so that the dyeing appears dark purple or dull-reddish under the lamp. With very pale shades some fluorescence from the substratum is visible, but it is modified by the colour of the dye itself. A greenish-yellow colour which is not fluorescent if dyed on wool in heavy shades will appear dirty violet-brown, and in light shades a weak greenish shade, whereas a blue dyestuff which appears inert in strong shades, in a weak shade will often appear pale blue owing to the blue fluorescence of the wool which is transmitted through the blue dyestuff.

In general, browns, greens and blacks and also many of the reds, violets or blue tones show no fluorescence on most materials, but a number of pink, orange, and yellow dyestuffs are fluorescent.

To examine dyed fibres in the unwoven state they should be combed parallel and smoothed flat as in ordinary colour matching, and if in the form of a textile the piece should be examined with the eye both at right angles and at very oblique angle to the surface, as the latter operation helps to eliminate any reflected violet or red rays passing through the filter.

The dyestuff may be dyed on as many materials for which it has an affinity; or it may be dissolved in water or in alcohol (1 : 200, 1 : 20,000, 1 : 200,000 parts), as these solutions often show the same fluorescence as the dyed fabric. If the dyestuff is already on the fabric it may be extracted with a suitable solvent, *e.g.*, alcohol for basic colours and ethylene diamine, pyridine or cyclohexanol for vat colours.

Certain dyes fluoresce on the fibre but not in solution, *e.g.*, ponceau brilliant and azorubin-S. This work has been extended

by H. Kautsky, A. Hirsch and W. Baumeister [89] to adsorption media such as silica gel, aluminium hydroxide and albuminoids, and they have obtained some very striking fluorescence effects which are acid-sensitive in the case of inorganic adsorbents only (see Photograph No. 10, p. 400).

Derivatives of quinoline, acridine, phthalein, primuline and thioflavin generally have a strong and characteristic fluorescence in aqueous or alcoholic solutions. Dyestuffs containing a S-group or a thiazole ring also show a very strong fluorescence.

H. R. Hirst [90] mentions that many benzene-substitution products and azo-dyes containing benzene nuclei show no fluorescence, whereas similar naphthalene compounds and dyes fluoresce strongly. L. Kummerer,[91] P. Balavoine [92] and P. Sisley [93] have classified and tabulated the fluorescence on fibres of over 1200 dyes, the intensity of those of the fluorescein class being particularly great.

P. Mougeot,[36] G. Martin [37, 38] and M. Déribéré [39] are other workers who have tabulated the fluorescence of a large number of dyestuffs, but it may be stated fairly definitely that the lamp cannot give an absolute identification of a particular dyestuff in our present state of knowledge. One of us (J. A. Radley) has found the lamp of value as a confirmatory test when the possibilities have been narrowed down (by chemical tests) to a few dyestuffs only, the capillary strip method and the adsorption method giving particularly useful information (see B. Bugyi [28]). As the fluorescence of certain dyestuffs varies according to the solvent it is possible to obtain some additional information in this way, but unfortunately the variations are on the whole not sufficiently great to allow two closely-related colours to be differentiated with any degree of certainty.

We may now pass on to a brief discussion of the behaviour of the more important classes of dyestuffs.

Basic Colours.—The most brilliantly fluorescent colours of this type are obtained from the reds, yellows and oranges, and they are most intense on wool and silk. The fluorescence on cotton is dull, as they are then dyed on a tannin-antimony mordant or an artificial mordant of the Tanninol BM or Katinnol-O type.

Rhodamine BS (C.I. No. 749) and especially Auramine OS (C.I. No. 655) dyed on a Tanninol mordant are readily distinguished from dyeings on a tannin-antimony mordant, as the

former are very much duller. The dyeings on the tannin-antimony mordant are in turn less fluorescent than when cotton is padded with the dyestuffs, or when these are dyed on wool or silk. Acridine Orange RS (C.I. No. 792) and Acronol Yellow TS (C.I. No. 815) also fluoresce brilliantly, whereas Safranine TS (C.I. No. 841), Chrysoidine YS (C.I. No. 20), Magenta PS (C.I. No. 677), Methyl Violet 2 BS (C.I. No. 680) and 10 BS (C.I. No. 681), Methyl Violet RS (I.C.I.) and Victoria Blue BS (C.I. No. 729) and RS (C.I. No. 728) show a very much less fluorescence, which is brighter when the dyeings are in the region of 0·2-0·5 per cent. on wool. The violet and blue dyestuffs are practically non-fluorescent in heavy shades on wool, silk or cotton. Dyeings on acetate silk behave in a similar manner to those on wool or silk.

It would appear that "loading" the molecule by means of a mordant decreases the excitability of the whole molecule, and in dealing with cotton substantive dyestuffs we find that a similar effect is obtained by loading the molecule by "after-coppering," while D. A. Derritt-Smith [102] finds that a copper-chrome after-treatment of fluorescent sulphur-colour dyeings reduces the fluorescence considerably.

Substantive or "Direct" Dyestuffs.—Most of these dyestuffs on the fibre, even including many of the yellows, show little or no fluorescence. Chlorazol Yellow 2 GS (C.I. No. 813), Primuline AS (C.I. No. 812) and Dianil Yellow 3 GN, however, show an intense yellow, yellow and green fluorescence, respectively, the intensity being similar to that of Auramine OS.

Chlorazol Pink YS (C.I. No. 225) and other derivatives of dehydrothiotoluidine containing a thiazole ring, such as Diamine Rose BD (C.I. No. 128) and Thiazine Red GXX, have a fairly strong red fluorescence on cotton in medium and deep shades. As the strength of the dyeing is decreased, however, the intensity of fluorescence is diminished and that of the substrate predominates progressively. Congo Red (C.I. No. 370) and Oxamin Red BN also have a red fluorescence, while Chlorazol Fast Yellow BNS (C.I. No. 814) and FGS (C.I. No. 622), Thioflavin S (C.I. No. 816) and its derivative Oxamin Yellow 3G have strong yellow fluorescence colours under the lamp.

The intensity of fluorescence of direct dyestuffs is generally strongest on wool and silk, followed by viscose and cotton in

order of decreasing intensity. A mixture of Thioflavin S (C.I. No. 816) with certain of the colours of the Soluble Blue type appears green, and a similar effect can be obtained by the use of Immedial Yellow GG (C.I. No. 955) instead of Thioflavin S.

The dyeings are best examined in strengths of 0·4 per cent. on wool or silk, and somewhat stronger on cotton, as in heavy shades the fluorescence colours are much less marked except in the case of the fluorescent yellow and red shades.

Treatment of Chlorazol Sky Blue FFS, Dianil Blue G (C.I. No. 508) and Chlorazol Blue RWS (C.I. No. 512) with copper sulphate decreases the fluorescence, but this is not sufficiently marked to allow the method to be used for the detection of "coppered" dyeings, and this also applies to formaldehyde-treated dyestuffs.

One of us (J. A. Radley) has found that when a direct dyestuff is developed a characteristic change may be noted in its fluorescence, and with Primuline AS (C.I. No. 812) on cotton the following fluorescent colours are observed: (*a*) alone, brilliant fluorescence, greener in shade than in daylight; (*b*) hypochlorite, yellow; (*c*) phenol, gold; (*d*) resorcinol, practically no fluorescence; (*e*) β-naphthol, red; (*f*) *m*-phenylene diamine, no fluorescence.

Azoic Colours.—Certain naphthols and their derivatives, such as Naphthol AS, which is the anilide of β-oxynaphthoic acid,[98] give characteristic fluorescence effects under the lamp which, because the influence of *p*H value on the shade, can be used to identify some of them.[40]

The developed azoic dyestuffs generally appear dark under the lamp, with the exception of Brenthol AS combinations with Fast Scarlet G, GG and RC bases [98] which produce red tones. Destruction of the dyestuff by spotting the patterns with nitric acid, stannous chloride and hydrochloric acid or with sodium hydrosulphite and ammonia gives no useful information as to the combination used. Derritt-Smith [102] finds that if the colour is reduced with hydrosulphite and caustic soda solutions and the pattern washed and examined under the lamp, then naphthol AS-G combinations give a reddish-blue fluorescence, and Naphthol AS-LG and AS-L3G combinations give greenish-yellow tones. Brown azoic combinations from Brenthol BT can be distinguished from those from Brenthol DA and FC by extracting the dyestuff with glacial acetic acid and reducing it by boiling

sodium hydrosulphite solution. On pouring on to a filter paper and rinsing with sodium hydroxide (60° Tw.) solution, Brenthol BT gives a blue, Brenthol DA greenish-yellow, and Brenthol FC a yellow fluorescent solution.

After dyeing packages or yarn with azoic colours C. M. Whittaker [99] examines them in ultra-violet light prior to soaping; the appearance of fluorescence indicates that the development is not complete, and this would lead to unevenness of dyeing when the package or yarn is soaped. This method is also applied to Soledon or Indigosol dyeings.

Vat Dyestuffs.—Several of the vat yellows fluoresce on silk, the most noteworthy being Caledon Yellow 5GS (I.C.I.) and Indanthrene Yellow GK, while on cotton Caledon Yellow 3GS and 5GS, Anthraflavin G (C.I. No. 1095) and RR (C.I. No. 1095) and Hydron Yellow G (C.I. No. 1159) are strongly fluorescent.

Caledon Red X5BS, Caledon Red Violet 2 RNS (C.I. No. 1161), Indanthrene Brilliant Violet 2R (C.I. No. 1104), Caledon Brilliant Violet RS (C.I. No. 1135), Caledon Blue GCS (C.I. No. 1113) and RS (C.I. No. 1106) and Brilliant Blue 3GS are fluorescent on silk, the three blues in 20 per cent. shades and the two violets having a similar fluorescence, so that no differentiation is possible.

Anthra Scarlet GG (C.I. No. 1228), Anthra Brilliant Green 5G and many of the thioindigoid vat colours are fluorescent when examined on cotton, the shade of the fluorescence becoming bluer as the tone of the dyestuff in daylight becomes more blue.

Solutions in benzene, pyridine or cyclohexanol of certain vat dyes, *e.g.*, Durindone Pink FF and Durindone Red B (C.I. No. 1207) and 3B (C.I. No. 1212), Caledon Purple R, 2R and 4R are strongly fluorescent even in daylight, and under the ultra-violet lamp the fluorescence colours are brilliant. On the whole, no matter what the shade is in daylight the fluorescence in solution under the lamp is generally yellow-red or orange to blood red, and cannot be used to distinguish one colour from another or in admixture (J.A.R.).

If the vat colours are reduced to the leuco-state by the use of an alkaline hydrosulphite, the solutions are non-fluorescent, owing probably to the fact that the reagent absorbs the ultra-violet light strongly and, in addition, can act as a deactivator of fluorescence.

If, however, the leuco-compounds are extracted from the

alkaline solution by means of ether or ethyl acetate those of the thioindigoids give yellow solutions showing a striking green fluorescence, which changes to blue when shaken with sulphuric acid. E. Herzog,[41] who made the above observations, finds that indigo does not show this change in fluorescence and further, points out that the red coloured benzanthraquinone compounds are fluorescent. We find, however, that Indigo LL (C.I. No. 1177) extracted from the vatted solution with ethyl acetate gives a brilliant blue fluorescence when shaken with sulphuric acid, but does not show a green fluorescence originally.

J. A. Radley has examined the leuco-esters of vat dyestuffs (Soledon colours) by means of the capillary strip method, and finds that nearly all of them give a number of characteristic zones, some of brilliant intensity and of varied colours. An alkaline solution of Soledon Jade Green XS gives a capillary strip which in visible light has only one zone (which is an orange pink in colour), although under the lamp the strip shows a deep orange zone followed by yellow, green and purple zones. Soledon Grey BS shows one pale olive-green zone in daylight, and puce, pale blue-grey, dull yellow, brown and bright lilac zones in ultra-violet light.

Acid Dyestuffs.—On wool practically all of the yellow acid dyestuffs with the exception of Tartrazine NS (C.I. No. 640) and Quinoline Yellow AS (C.I. No. 801) appear deep greenish or dirty olive brown in colour, whilst a number of the reds show some fluorescence and appear lighter under the lamp than in daylight. Certain reds, however, appear nearly black even in 1 per cent. dyeings.

The various halogenated phthalein derivatives, *e.g.*, Erythrosine (C.I. Nos. 772, 773) and Rose Bengal brands (C.I. Nos. 777 and 779) and Phloxine B (C.I. No. 778), Eosine Y (C.I. Nos. 768 and 771) are strongly fluorescent. The bluish-red fluorescence of Acid Magenta AS (C.I. No. 692) allows it to be distinguished from Coomassie Violet 2RS (C.I. No. 758), which has a bright scarlet-red fluorescence, which is, however, more intense than the former when dyed on silk. On silk the fluorescence of Naphthalene Scarlet BS is outstanding among the red dyestuffs of the acid range.

Mordant Colours.—These are generally non-fluorescent on wool or silk, although when applied without a mordant some show

fluorescence. J. A. Radley has found that Alizarine Orange AS is yellow-red and Alizarine Red AS (C.I. No. 1034) is bright red when dyed by the chromate method, but if applied by the after-chrome method there is no fluorescence, so that the dyeing method employed may be determined. Solochrome Red 6 BS has a violet fluorescence irrespective of the method of application. The acid dyestuff, Coomassie Milling Scarlet 5 BS, which is not normally regarded as a mordant colour but which is employed in conjunction with chrome dyestuffs, decreases in fluorescence when dyed by the after-chrome method, but this change is not so marked as with Coomassie Milling Scarlet GS (C.I. No. 443) which is another dyestuff of this type.

Acetate Rayon Dyestuffs.—The anthraquinone series of acetate silk dyestuffs contains a number which are fluorescent, some being fairly intense, whereas the azo-type are inert, often appearing almost black under the lamp in heavy shades.

The fluorescence is shown chiefly by the yellows and reds of the anthraquinone series, and among the violets, blues and blacks only Duranol Violet 2RS fluoresces, appearing light violet in weak shades and reddening progressively in heavier dyeings until, in 5 per cent. shades, it appears blood red. It should be noted that the water-soluble type of azo-dyestuffs used in colouring acetate rayon are also non-fluorescent.

Dyestuffs with a green fluorescence are obtainable from phenyl pyridine compounds,[29] and the fluorescence of eleven sets of symmetrical cyanine dyestuffs has been recorded by N. I. Fisher and F. M. Hamer.[47] Well-defined crystalline compounds of meta-phosphoric acid and the triphenyl methane dyestuffs which have a strong fluorescence have been examined by H. Hermann,[48] and other relevant papers occur in the references.[49]

Accelerated Fading Tests.—The *fading of dyestuffs on fibres* is dealt with fully under Paper (p. 344) and Textiles (p. 360), but little work has been carried out on solutions of the dyes themselves other than that described in Chapter V on the changes in fluorescence which occur on irradiation. G. A. Bravo,[94] however, attempted to obtain quantitative measurements of fading by plotting the percentage of light reflected against the wave-lengths, using filters (4850 to 6000 Å.), and was able to show an approximate correspondence between the mercury and carbon arc lamps.

J. Grant [50] also has examined the fading of dyestuffs on paper and has compared the results obtained with the carbon arc, mercury vapour and daylight. This worker pointed out that the rate of change of fluorescence might be used in many cases as a criterion of fading (see p. 344), and J. A. Radley has found that on textiles the fluorescence of a fluorescent dyestuff is often destroyed just before the fading become visible to the eye in ordinary daylight; the test certainly often appears to accentuate the difference in fluorescence between the exposed and unexposed portions, although in weak shades (*e.g.*, on wool) the simultaneous destruction of the fluorescence of the substrate may play an important part in this change. Further work has been carried out by G. A. Bravo,[51] and also by P. Mougeot,[52] who considers that the Toussaint photo-colorimeter gives good results when used to measure the extent of the fading (see also G. R. Fonda [53]). The lamp has also been suggested [54] as a means of following the effects of washing on dyed textiles.

The Detection of Faults.[95]—W. Sieber and J. Kasche [96] and O. Gaumnitz [97] employed the lamp for the detection of threads which were not evenly dyed. In daylight the colour of a length of thread may appear uniform, but on weaving the inequalities in the dyeing become apparent, and may lead to rejection of the cloth or to a great diminution in its value. It is also claimed that the use of the lamp facilitates recognition of the use of mixed colours (see also Photographs Nos. 6, 8 and 10, and J. Muir [54]).

O. Mecheels [55] examined the fluorescence of Naphthol-*AS* by spotting an aqueous solution on bleached cotton fabric and examining in both transmitted and reflected ultra-violet lights. Pure aqueous Naphthol-*AS* appeared white, and its sodium and potassium salts yellow. In the presence of sodium carbonate or ammonia, only a blue-white fluorescence was obtained, and in alcoholic potassium hydroxide solution, a deep yellow (by reflected light) or brown (by transmitted light). Naphthol-*AS* is usually made up with sodium hydroxide, formalin, and turkey-red oil, and cotton immersed in this mixture and dried shows a bright yellow or dark blue to green colour at the boundary between the immersed and untreated parts. If the sodium hydroxide is replaced in this case by potassium hydroxide, the blue band is less pronounced and is absent if an alcoholic solution of potassium hydroxide is used. In this way, *water spots*

produced in dyeing may be detected by treating with Naphthol-*AS* and examining in ultra-violet light.

In order to distinguish between dyeings with Naphthol-*AS* or similar dyes and developed direct dyes (on cotton), the cotton is fully discharged in a boiling solution containing 2·4 per cent. of sodium hydrosulphite and 3·6 per cent. of sodium hydroxide (*d.* 1·385), and placed while wet on a filter paper which is dried in air and examined in ultra-violet light. The latter dyes produce a white to pale violet fluorescence on the paper and cotton, whilst the former (except Naphthol-*ASG*) produce a yellow fluorescence on the cotton and none on the paper.

The action of light and of washing on the dyed textile has been followed by W. D. Appel [56] by means of a Marten photometer which enabled him to compare the relative brightness of the fluorescence after it had passed through colour filters allowing the passage of light of one wave-length only.

A further use to which C. M. Whittaker [99] puts the lamp is for the matching of lingerie shades, as it is essential that the matching should fluoresce similarly to the standard material or they may not give the same effect in twilight.

The lamp may also be used to detect the marking-off of colours, either in the ager or during washing. The marking-off is probably most readily observed with the insoluble type of anthraquinone acetate silk colours, although generally this is apparent quite readily in daylight; in some cases, however, a short steaming will show no apparent marking-off in daylight although this can be detected under the lamp, and longer steaming or poor steaming conditions will lead to visible marking-off of these dyestuffs. H. R. Hirst [101] describes applications to the identification of dyeing faults on wool; in particular, iron due to the use of an excessive quantity of catalyst to accelerate peroxide bleaching baths is associated with mineral oil, and produces fluorescent spots.

The examination of the weakening of shade on washing a dyed textile has already been mentioned, and this applies to all fluorescent dyestuffs; marking-off during washing (*e.g.*, of vat colours in the presence of alkali and reducing substances, such as starches, glucose or dextrins used in the finishing agents or printing thickeners) is sometimes detectable. The marking-off of basic colours on acetate silk is also detectable by this means.

The detection of oil or grease spots is mentioned on p. 369.

Miscellaneous.—Some special uses of the lamp are in the production of stage and scenic effects and for special window displays, and much ingenuity and money has been expended in this direction in many countries. M. Déribéré [57] has dealt with the production of fluorescent effects on wood and shellac borax lacquers, and nitrocellulose lacquers or Parallacs are very suitable media for the colouring of wood, cardboard or papier-maché for this type of work; in some cases alcoholic solutions of dyestuffs can readily be applied to give effective results.

C. Paine and J. A. Radley [58] have claimed the use of cotton-substantive and colourless compounds having a strong blue fluorescence in ultra-violet light for use in this type of work. Another specialised use to which the lamp is sometimes put is in connection with light-sensitive dyestuffs to obtain photographic prints [59] on materials, but the output then suffers to such a degree as to render the process uneconomical for long runs or except where a high price is obtainable.

The titration of dyestuff solutions is facilitated by the use of the lamp, and fluorescent indicators are fully dealt with on pages 310-318. Dyes are further discussed in Chapter XVIII, on Textiles; see also page 339, and Photographs Nos. 6 and 10 (facing p. 400); F. G. H. Macrae [105] of the Theatre Royal, Drury Lane, also mentions the use of the fluorescence of auramine, eosin and fluorescein in producing stage-effects. For plant colouring matters see Chapters III and XIV.

REFERENCES.

1 V. L. Levschin, *Compt. rend. U.S.S.R.*, 1934, 2, 405; *Acta Physicochim.*, 1935, 1, 685.

2 G. Braun, *Jour. Chim. Phys.*, 1936, **33**, 559.

3 A. Wrzesińska, *Acta phys. Polon.*, 1936, 4, 475; *Chem. Zentr.*, 1938, **109**, (i), 2525.

4 A. Wrzesińska, *Bull. acad. Polonaise*, 1935, A, 38; *ibid.*, 1936, 10A, 568.

5 J. Bouchard, *Jour. Chim. Phys.*, 1936, **33**, 127.

6 C. Stora, *Compt. rend.*, 1936, **202**, 48, 408.

7 P. Fröhlich, *Acta Lit. Sci. Univ. Francisco, Josephine Sect. Chem. Min. Phys.*, 1934, 4, 1.

8 F. G. Wick and C. G. Throop, *Phys. Rev.*, 1935, **47**, 329.

9 W. Szymanowski, *Zeit. Physik*, 1935, **95**, 440, 466.

10 W. Szymanowski, *ibid.*, 1935, **95**, 450.

11 W. Szymanowski, *ibid.*, 1935, **95**, 460.

[12] F. Duschinsky, *Acta phys. Polon.*, 1936, **5**, 255 ; *Chem. Zentr.*, 1937, (ii), 2791.
[13] A. Jabloński, *Zeit. Physik*, 1935, **95**, 53.
[14] J. C. Ghosh and S. B. Sen-Gupta, *Zeit. phys. Chem.*, 1938, **41B**, 117.
[15] B. J. Svenschnikov, *Jour. Phys. Chem. U.S.S.R.*, 1934, **5**, 38, 45 ; *Brit. Chem. Abst. A*, 1937, **1**, 11.
[16] B. J. Svenschnikov, *Acta Physicochim. U.S.S.R.*, 1936, **4**, 453.
[17] F. S. Barishanskaja, *Compt. rend. Acad. Sci. U.S.S.R.*, 1938, **19**, 465.
[18] V. L. Levschin, *Acta Physicochim. U.S.S.R.*, 1936, **4**, 221.
[19] G. R. Fonda, *Jour. Opt. Soc. Amer.*, 1936, **26**, 316.
[20] P. Pringsheim, *Angew. Chem.*, 1936, **49**, 648.
[21] C. Achard, A. Boutaric and J. Bouchard, *Compt. rend.*, 1935, **201**, 629.
[22] K. Weber, *Zeit. phys. Chem. U.S.S.R.*, 1935, **30B**, 69.
[23] J. Bouchard, *Jour. Chim. Phys.*, 1936, **33**, 325.
[24] H. Hellström, *Naturwiss.*, 1936, **24**, 76.
[25] K. Weber, *ibid.*, 1936, **24**, 318.
[26] L. Grisebach, *Zeit. Physik*, 1936, **101**, 13.
[27] P. Pringsheim and H. Vogels, *Acta phys. Polon.*, 1936, **4**, 331, 341 ; *Chem. Zentr.*, 1937, **108**, (ii), 1985.
[28] B. Bugyi, *Kolloid Ztg.*, 1938, **84**, 74.
[29] I. G. Farbenind., E.P. 445,781 ; equiv. to F.P. 779,901, 1934.
[30] A. Jabloński, *Acta phys. Polon.*, 1936, **4**, 371, 389.
[31] D. R. Morey, *Text. Res.*, 1934, **5**, 105.
[32] S. M. Mitra, *Zeit. Physik*, 1934, **91**, 61.
[33] S. J. Wavilow and A. N. Sevtsch, *Compt. rend. Acad. Sci. U.S.S.R.*, 1936, **3**, 271, 277.
[34] W. Kessel, *Zeit. Physik*, 1936, **103**, 125.
[35] S. J. Golub, *Phys. Zeit. Sovietunion*, 1935, **7**, 49 ; through *Brit. Chem. Abst. A*, 1936, 271.
[36] M. P. Mougeot, *Rév. Gén. Mat. Col.*, 1936, **40**, 1.
[37] G. Martin, *ibid.*, 1938, **42**, 8.
[38] G. Martin, *ibid.*, 1938, **42**, 81.
[39] M. Déribéré, *Rusta*, 1936, **11**, 219, 221.
[40] Anon, *T.I.B.A.*, 1937, **15**, 496.
[41] E. Herzog, *Rév. Gén. Mat. Col.*, 1933, **37**, 284.
[42] S. B. Sen-Gupta, *Jour. Indian Chem. Soc.*, 1938, **15**, 263.
[43] J. Weiss, *Trans. Faraday Soc.*, 1936, **32**, 1331.
[44] J. Weiss, *Nature*, 1935, **136**, 794.
[45] H. v. Euler, K. Brandt and H. Hellström, *Naturwiss.*, 1935, **23**, 794.
[46] K. Weber, *ibid.*, 486.
[47] I. N. Fisher and F. M. Hamer, *Proc. Roy. Soc.*, 1936, **A154**, 703.
[48] H. Hermann, *Microchim. Acta*, 1938, **3**, 193.
[49] G. Scheibe, *Kolloid Zeit.*, 1938, **82**, 1.
[50] J. Grant, *Paper Trade Rev.*, 1933, **100**, 1866 ; *Chem. Age*, 1933, **29**, 4.
[51] G. A. Bravo, *Ind. Chim.*, 1934, **9**, 313, 461.
[52] P. Mougeot, *Bull. Soc. franc. elec.*, 1935, **5**, 965.
[53] G. R. Fonda, *Jour. Opt. Soc. Amer.*, 1936, **26**, 316.
[54] J. Muir, *Ind. Chem.*, 1932, **8**, 348.
[55] O. Mecheels, *Textilber.*, 1931, **12**, 581.
[56] W. D. Appel, *Amer. Dyestuff Reporter*, 1928, **17**, 49.
[57] M. Déribéré, *T.I.B.A.*, 1937, **15**, 145.
[58] I. C. I., C. P. Paine and J. A. Radley, U.S.P. 2,089,413.
[59] H. Freytag, *Photo. Korr.*, 1935, **71**, 52.

[60] A. V. Banov, *Zeit. Phys.*, 1929, **58**, 811.
[61] J. Bouchard, *Compt. rend.*, 1934, **198**, 649 ; **199**, 43, 460.
[62] F. Perrin, *ibid.*, 1924, **178**, 1928.
[63] P. Balovoine, *Arch. Sci. Phys. Nat.*, 1930, **5**, 62.
[64] M. Haitinger, *Mikrochem.*, 1932, **11**, 429.
[65] F. G. Wick and C. G. Throop, *Bull. Amer. Phys. Soc.*, 1934, **9**, 11.
[66] J. R. Jenness, *Phys. Rev.*, 1929, **34**, 1275.
[67] W. König and W. Regner, *Ber.*, 1930, **63B**, 2823.
[68] K. Ochai, *Bull. Chem. Soc. Japan*, 1930, **5**, 203.
[69] F. M. Frank and S. J. Wavilow, *Zeit. Phys.*, 1931, **69**, 100.
[70] S. Boudin, *Jour. Chim. Phys.*, 1930, **27**, 285.
[71] W. Szymanowski, *Polish Acad. Sci.*, 1925, Feb. 4.
[72] M. Phisalix, A. Boutaric and J. Bouchard, *Compt. rend.*, 1935, **200**, 976.
[73] A. V. Banov, *Journ. Phys. Chem. U.S.S.R.*, 1934, **5**, 46 ; through *Amer. Chem. Absts.*, 1934, **28**, 43.
[74] Y. Cauchois, *Jour. Chim. Phys.*, 1930, **27**, 336.
[75] E. Merritt and D. Morey, *Phys. Rev.*, 1930, **36**, 1386.
[76] J. Wawilow, *Phil. Mag.*, 1922, **43**, 307.
[77] J. Wawilow, *Zeit. f. Phys.*, 1924, **22**, 266.
[78] J. Wawilow, *ibid.*, 1925, **31**, 750.
[79] J. Wawilow, *ibid.*, 1925, **32**, 721.
[80] J. Wawilow and W. L. Levschin, *Phys. Zeit.*, 1922, **23**, 173.
[81] J. Wawilow and W. L. Levschin, *Zeit. f. Phys.*, 1923, **16**, 135.
[82] E. L. Nichols and E. Merritt, *Carnegie Inst. Publ.*, No. 152.
[83] A. Jabłoński, *Bull. Int. Acad. Polon. Math. Nat.*, 1934, **A**, 14.
[84] A. Jabłoński, *Zeit. f. Phys.*, 1935, **94**, 38.
[85] S. Mitra, *Indian Jour. Phys.*, 1933, 171 ; 1934, 445.
[86] M. Haitinger, *Mikrochem.*, 1935, **16**, 321.
[87] A. Seyewetz and J. Blanc, *Compt. rend.*, 1929, 188, 714 ; *Bull. Soc. Chim.*, 1929, **45**, 611.
[88] H. E. Fierz-David, *Jour. Soc. Dyers and Col.*, 1929, **45**, 133.
[89] H. Kautsky, A. Hirsch and W. Baumeister, *Ber.*, 1931, **64**, 2053.
[90] H. R. Hirst, *Journ. Text. Inst.* (*Special Issue*), 1927, **18**, 369.
[91] L. Kummerer, *Textilber.*, 1928, **9**, 415.
[92] P. Balavoine, *Arch. Sci. Phys. Nat.*, 1930, **5**, 62.
[93] P. Sisley, *Chim. e Ind.* (*Special Issue*), Mar., 1932, p. 508.
[94] G. A. Bravo, *Ind. Chim.*, 1934, **9**, 313, 461.
[95] A. E. Sampson, *Amer. Dyestuff Reporter*, 1935, **24**, 8.
[96] W. Sieber and J. Kasche, *Textilber.*, 1928, **9**, 234.
[97] O. Gaumitz, *ibid.*, 1926, **7**, 237.
[98] F. M. Rowe and Levin, *Jour. Soc. Dyers and Col.*, 1924, 218.
[99] C. M. Whittaker, Private Communication.
[100] P. Pringsheim and H. Vogels, *Jour. Phys. Radium*, 1937, **8**, 121.
[101] H. R. Hirst, *Jour. Soc. Dyers and Col.*, 1937, **53**, 45.
[102] D. A. Derritt-Smith, *Jour. Text. Inst.*, 1937 **28**, T145.
[103] L. Meunier and A. Bonnet, *Compt. rend.*, 1925, **180**, 2038 ; **181**, 465 ; *Jour. Intl. Soc. Leather Trades' Chem.*, 1925, **9**, 340.
[104] F. T. Addyman, *Dyer and Calico Printer*, 1929, **61**, 266.
[105] F. G. H. Macrae, *Amer. Dyestuff Reporter*, 1933, **22**, 647.

ADDITIONAL REFERENCES.

J. Wawilow, *Compt. rend. Acad. Sci. U.S.S.R.*, 1937, **16**, 255 (Polarised fluorescence of dyestuffs).

P. G. Glakhow and J. A. Khvostikow, *ibid.*, 259 (Polarised fluorescence of dyestuffs).

Anon, *Dyer and Calico Printer*, 1929, **61-62**, 153 (General).

C. M. Whittaker, *ibid.*, 1929, **61-62**, 44 (Considers that the influence of ultra-violet rays on fading has been over-stressed).

C. Stora, *Compt. rend.*, 1936, **202**, 48, 408, 2152 (Becquerel effect and photochemical sensitiveness of dyestuffs).

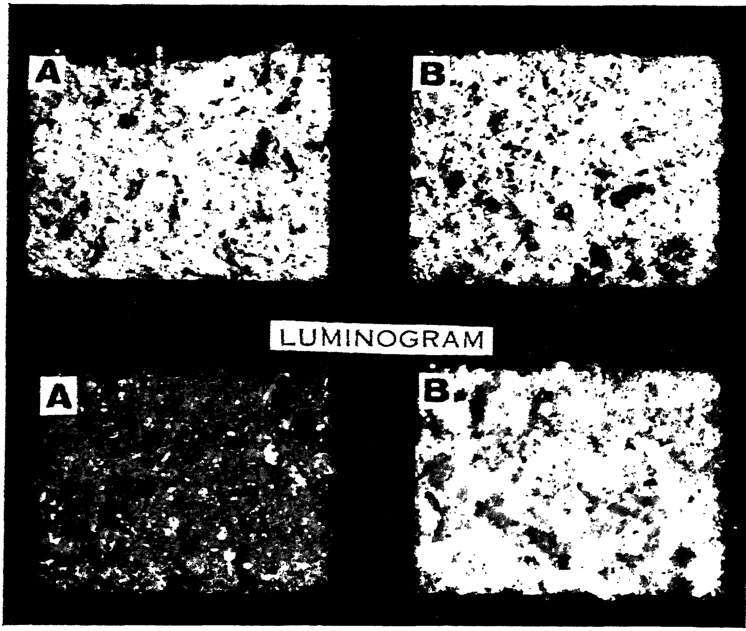
E. V. Schpolski and G. D. Sheremetiev, *Acta Physicochim.*, 1936, **5**, 575 (Quenching of fluorescence of dyestuffs).

S. W. Cram, *Zeit. Physik*, 1936, **103**, 551 (Decay of fluorescence of uranine).

E. E. Jelley, *Nature*, 1936, **138**, 1009 (Certain molecularly-dispersed dyestuffs fluoresce similarly to the ruby).

S. I. Wawilow, *Zeit. Physik*, 1927, **42**, 311 (Fluorescence of dyestuffs as function of wave-length of exciting light).

PHOTOGRAPHS

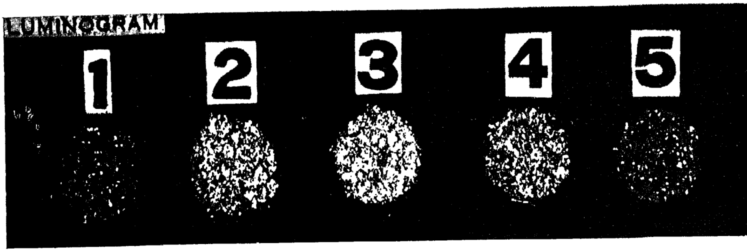


No. 1.—Wheat.

In the top row are ordinary photographs of wheat (A), and wheat containing 20 per cent. of tares (B). In the bottom row are the corresponding luminograms (the light blue fluorescence obtained in the former case is overshone in the latter case by the vivid golden-yellow due to the tares). (*See p.* 92.)

[*Taken by Lt.-Col. W. R. Mansfield.*

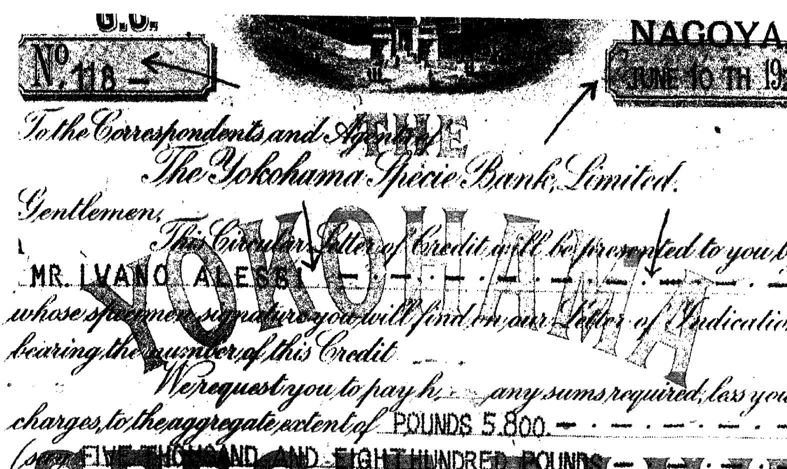
[*To face p.* 400.



No. 2.—Rhubarbs.

Luminograms of ground Rhubarbs supplied by the late Mr. E. T Brewis, F.I.C., taken by Lt.-Col. W. R. Mansfield). (*See p.* 129.)

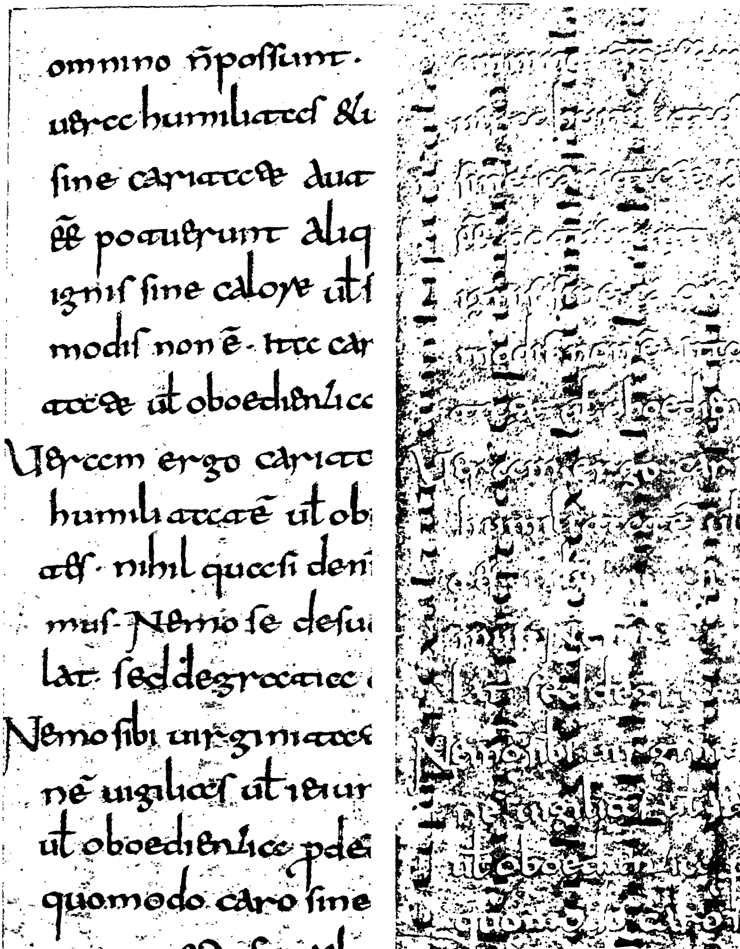
(1) *R. Palmatum.* (Brown, white striæ.)
(2) High-dried flats. (Purple, with yellow and white patches.)
(3) Rough rounds. (Similar, more intense.)
(4) *R. Rhaponticum.* (Similar, less intense.)
(5) Canton. (Purple to orange, with white veins.)



No. 3.—Forged Document.

The original erased signature is plainly visible in the Photograph. (*See p.* 226.)

[*By courtesy of The British Banc*



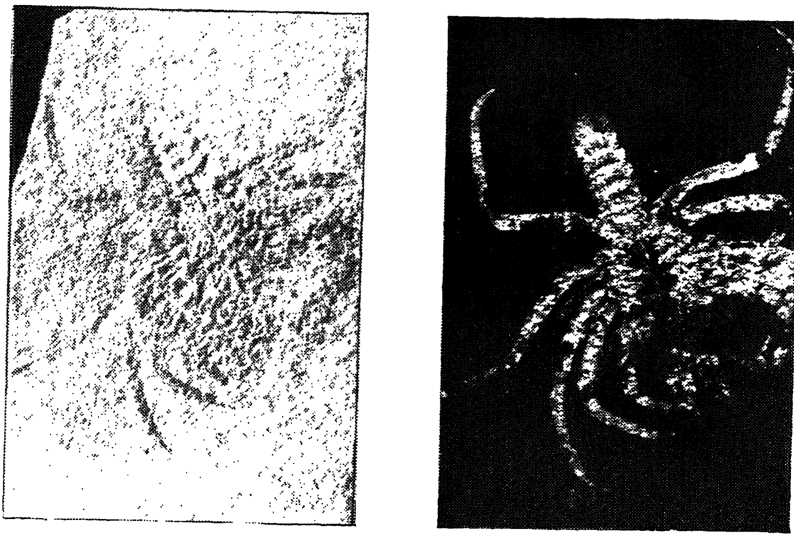
No. 4.—Palimpsest.

Left—Photograph in ordinary light.

Right—Photograph showing fluorescence of older writing underneath.

(*See p.* 278.)

[*By courtesy of Prof. Kögel and The British Hanovia Quartz Lamp Co. Ltd.*

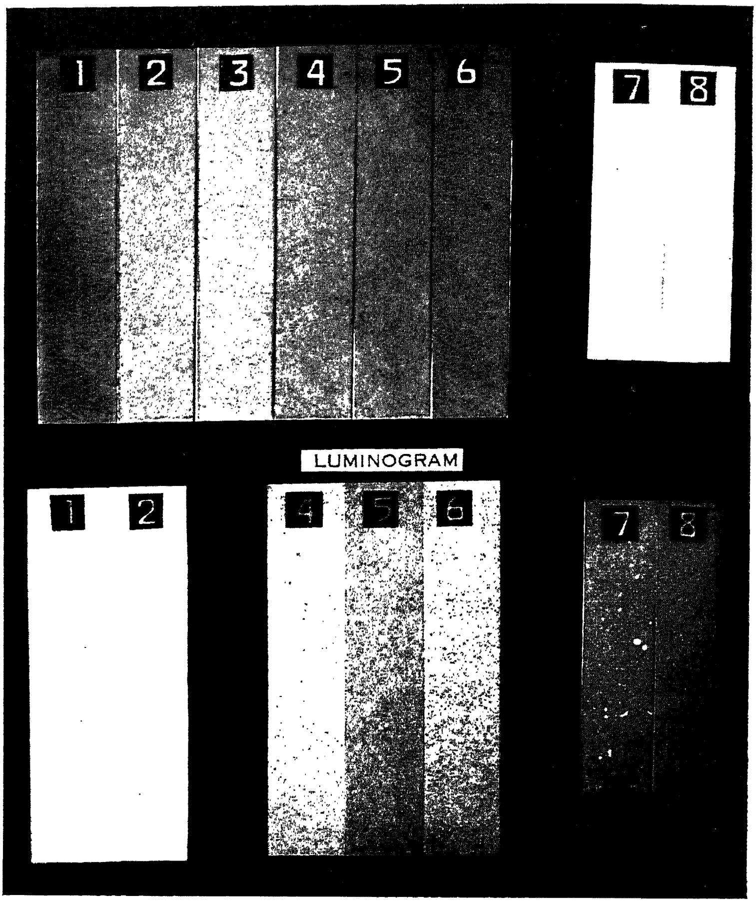


No. 5.—Fossils.

Left—Photograph in ordinary light.
Right—Photograph in ultra-violet light.

(*See p.* 284.)

[*By courtesy of Prof. Miethe and The British Hanovia Quartz Lamp Co. Ltd.*



No. 6.—PAPERS.

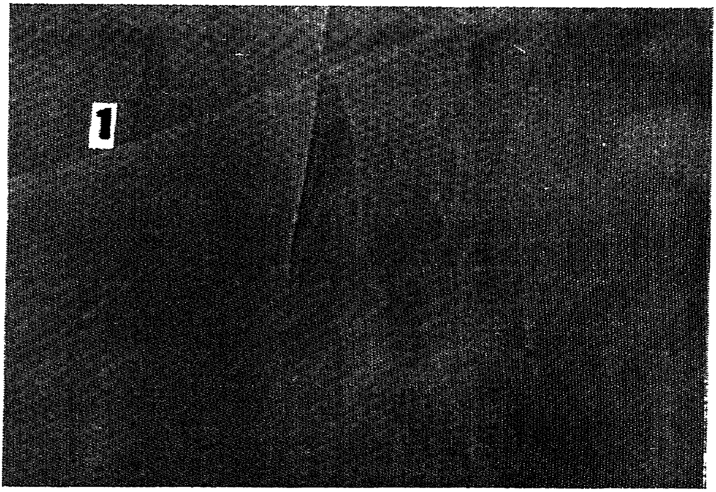
In the top row are photographs taken in ordinary daylight; in the bottom row are the corresponding photographs taken in ultra-violet radiation (by Lt.-Col. W. R. Mansfield).

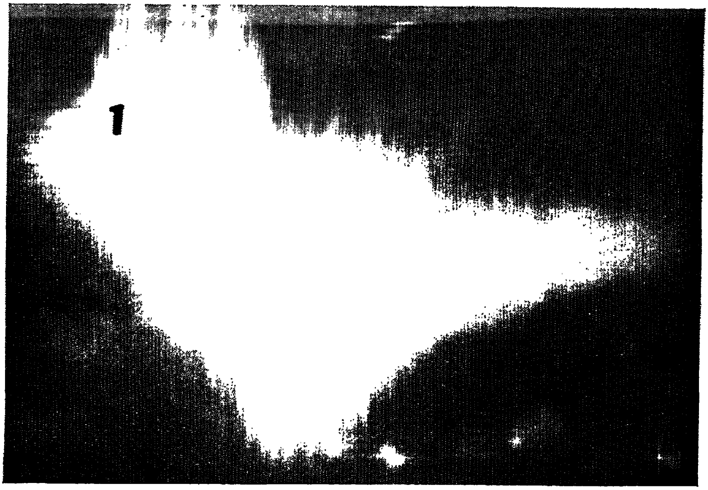
Nos. 1 to 4 are strips of paper containing different yellow dyes. They appear almost identical in ordinary light, but vary considerably in ultra-violet light, both in intensity and colour (golden, pale yellow, dark brown and yellow-grey, respectively).

Nos. 5 and 6 are the same as No. 4 after exposure for one week to sunlight, and for two hours to ultra-violet light, respectively.

No. 7 contains a wax size; the fluorescent particles of wax are plainly visible on comparison with No. 8 which contains no wax.

(*See pp.* 340, 343 *and* 344.)



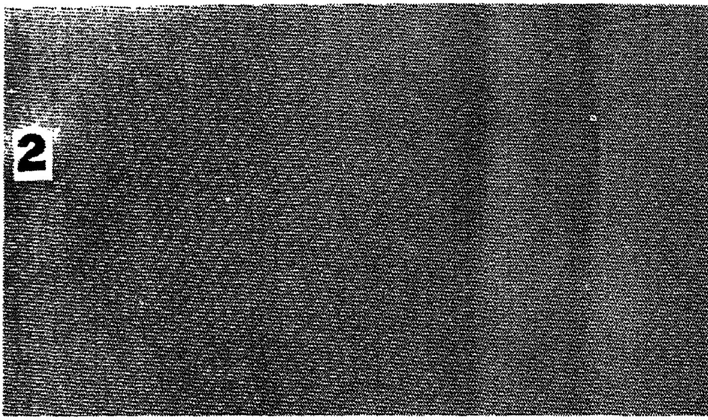


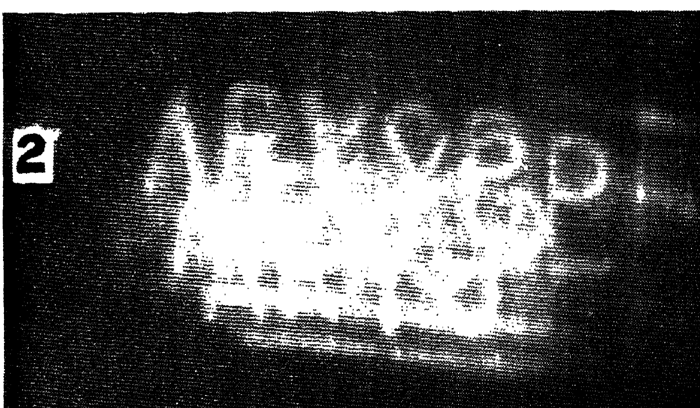
No. 7.—Freshly-spotted Oil on Viscose.

Top—Photograph in ordinary light.
Bottom—Luminogram.

(*See pp.* 369 *and* 371.)

[*By courtesy of "The Industrial Chemist" taken by Lt.-Col. W. R. Mansfield.*



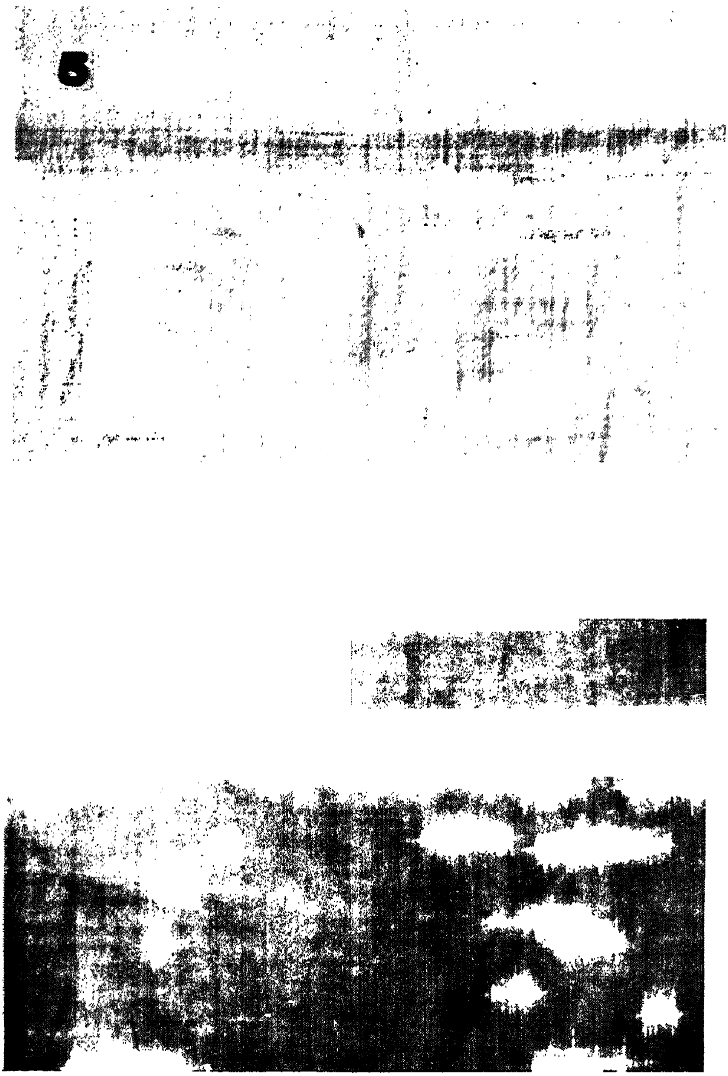


No. 8.—Marking by Contact of a Stamped Heading on Viscose Fabric.

Top—Photograph in ordinary light.
Bottom—Luminogram.

(*See pp.* 369 *to* 371.)

[*By courtesy of "The Industrial Chemist" taken by Lt.-Col. W. R. Mansfield.*

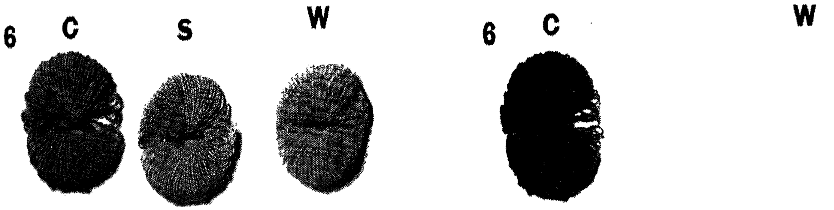


No. 9.—Mineral Oil introduced by Weaving in Weft and not Removed by Bleaching.

Top—Photograph in ordinary light.
Bottom—Luminogram.

(*See pp.* 369 *and* 371.)

[*By courtesy of "The Industrial Chemist"; taken by Lt.-Col. W. R. Mansfield.*

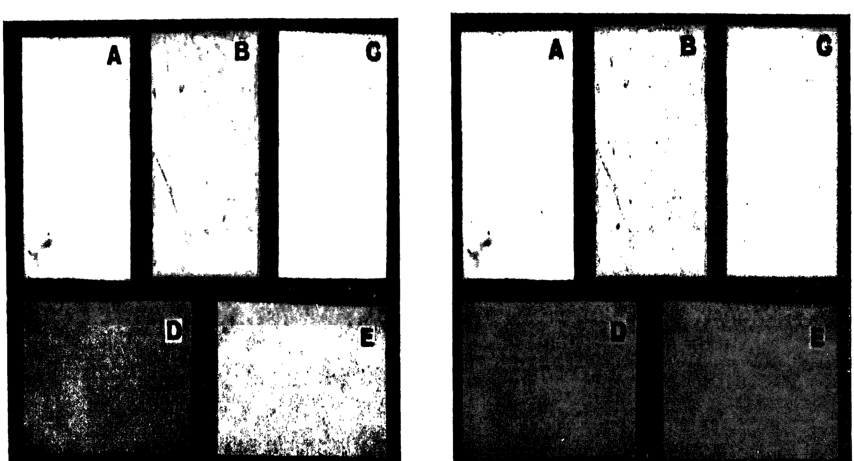


No. 10.—Dyed Textiles.

Left—Photograph in ordinary light of Rhodamine-B dyed on cotton with a mordant (C), on silk (S) and on wool (W).
Right—The corresponding Luminograms.

(*See pp.* 389 *and* 390.)

[*By courtesy of "The Industrial Chemist" taken by Lt.-Col. W. R. Mansfield.*



No. 11.—Wood Pulps.

A is a strong, bleachable sulphite pulp, and E is a bleached pulp, the others being intermediate varieties.
In ordinary light (*left*) there is no correlation between appearance and bleachability. In ultra-violet light (*right*) A has a strong violet fluorescence which gradually disappears in the series and is absent from E.

(*See p.* 336.)

[*Taken by Lt.-Col. W. R. Mansfield.*

No. 12.—Rye Grass.

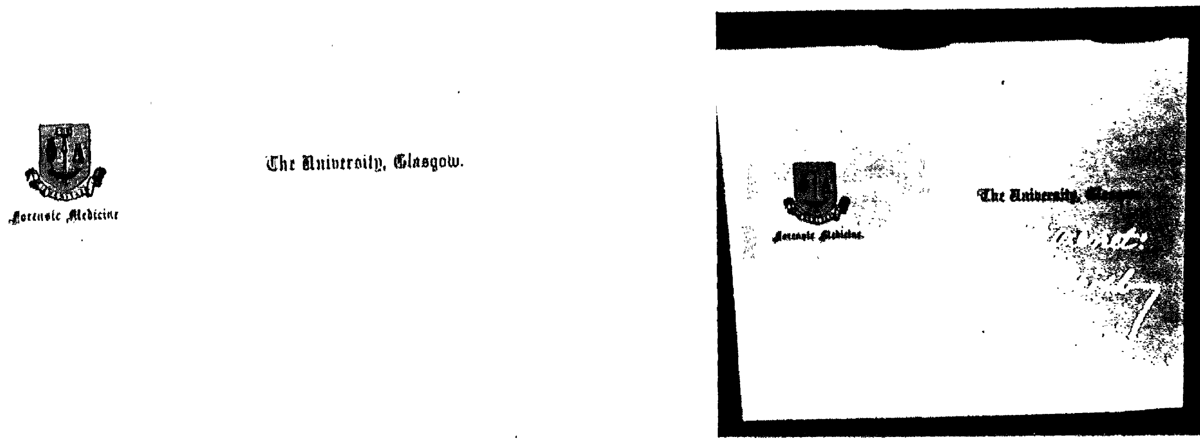
Left—Seedlings about 10 days old, germinated on filter-paper. True perennial rye-grass.

Right—The same, but Italian rye-grass. The paper adjacent to the roots has a blue fluorescence which appears white in the photograph.

Photograph taken in the light of a 12-inch mercury lamp, 3·5 amps., at 18 inches, with Ilford special panchromatic plates and "Q" filter; exposure 10 mins. at F/6·8.

(*See p.* 92.)

[*By courtesy of Prof. S. P. Mercer and P. A. Lineham.*



No. 13.—Invisible Writing in Urine.

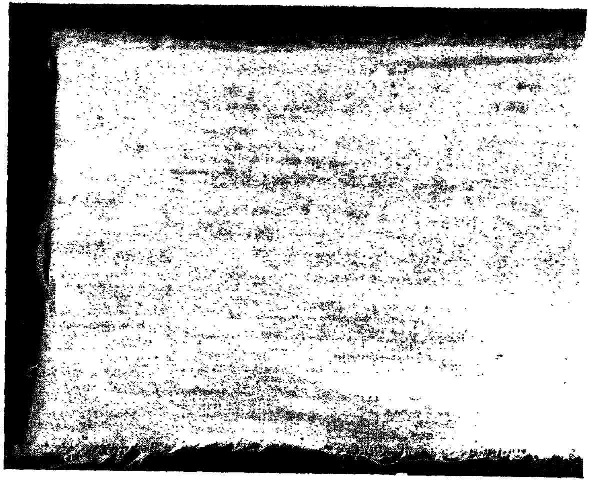
Left—In visible light.

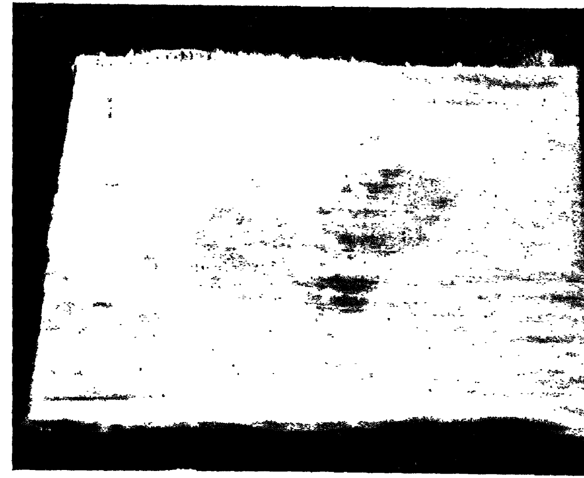
Right—In ultra-violet light.

Objects 7 ins. from filtered mercury arc. Lens filter, 1 per cent. cerium ammonium nitrate. Ordinary camera, stop F/11; exposure 15 to 25 mins.

(*See p.* 229.)

[*By courtesy of Dr. F. W. Martin.*





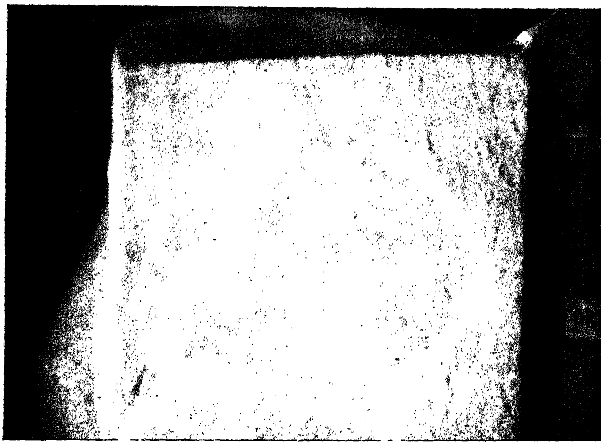
No. 14.—Bloodstains.

Photographs of washed bloodstained cloth.
Left—In visible light.
Right—In ultra-violet light.

(*See p.* 231.)

[*By courtesy of Dr. F. W. Martin.*

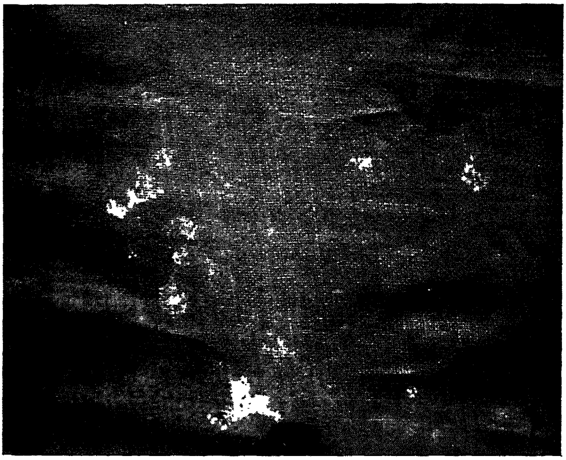
For photographic details, see No. 13.



No. 15.—SEMINAL STAIN.

Photograph in ultra-violet light. For details, see No. 13. (*See p.* 230.)

[*By courtesy of Dr. F. W. Martin.*

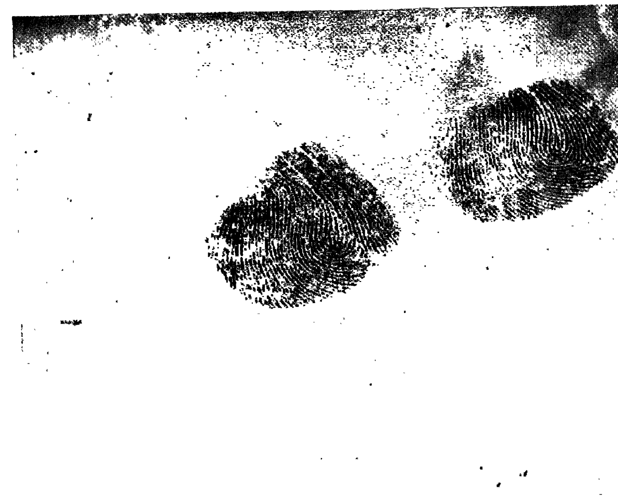


No. 16.—Mildewed Cloth.

No. 15 Wratten filter. Ilford "soft-gradation" panchromatic plate. Exposure 10 mins. for fluorescence, and 5 secs. in unfiltered light to obtain detail of fabric.

(*See p.* 371.)

[*Taken by J. M. Preston.*



No. 17.—Finger-Prints.

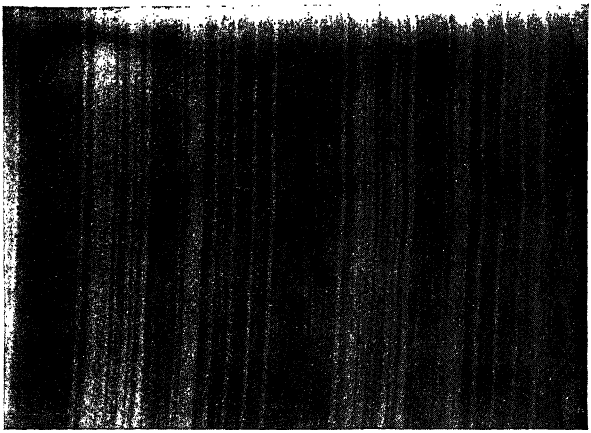
Left—Finger-prints on a lacquered tin box powdered with anthracene; photographed by incandescent electric light.

Right—The same, in ultra-violet light.

Object 40 cms. from mercury arc. Discharge 15 cms. long; current 3 amps. Wood's glass (1·5 cm. thick) for arc and 4 Andrews' Super-Protex filters (each 8 mm. thick) for lens. Focal length of lens, 7 ins.; aperture F/7; exposure 6 mins. "Sanderson" extension camera.

(*See p.* 229.)

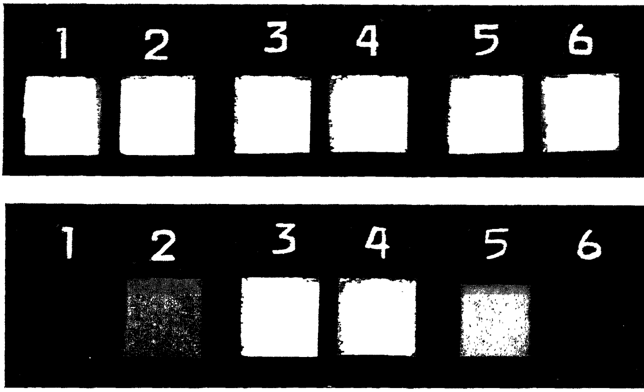
[*Taken by* C. H. Edlin.



No. 18.—STREAKY CLOTH.

(*See p.* 371.)

[*Taken by H. R. Hirst, and reproduced by courtesy of the Wool Industries Research Association.*



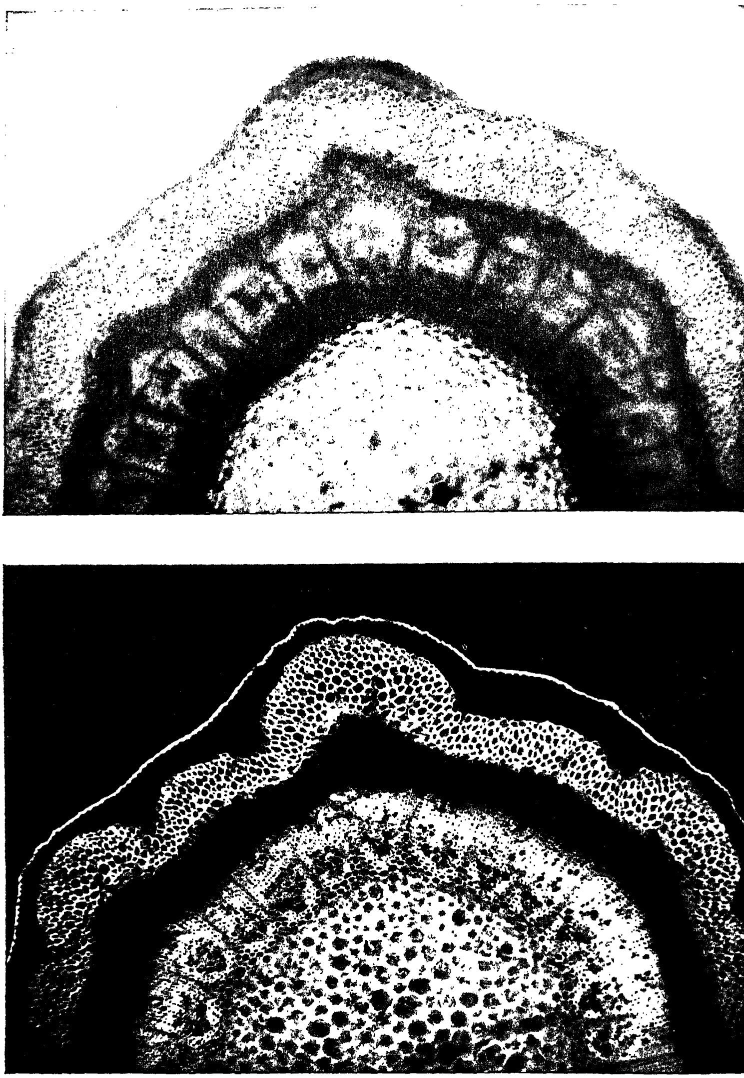
No. 19.—PIGMENTS AND LOADINGS.

Top row—In visible light.
Bottom row—In ultra-violet light.

(1) Good commercial china clay.
(2) The same, with a tinting colour.
(3) Zinc oxide, first grade.
(4) Zinc oxide, lower grade.
(5) Precipitated chalk, first grade.
(6) Precipitated chalk, lower grade.

(*See p.* 340.)

[*Taken by Lt.-Col. W. R. Mansfield.*



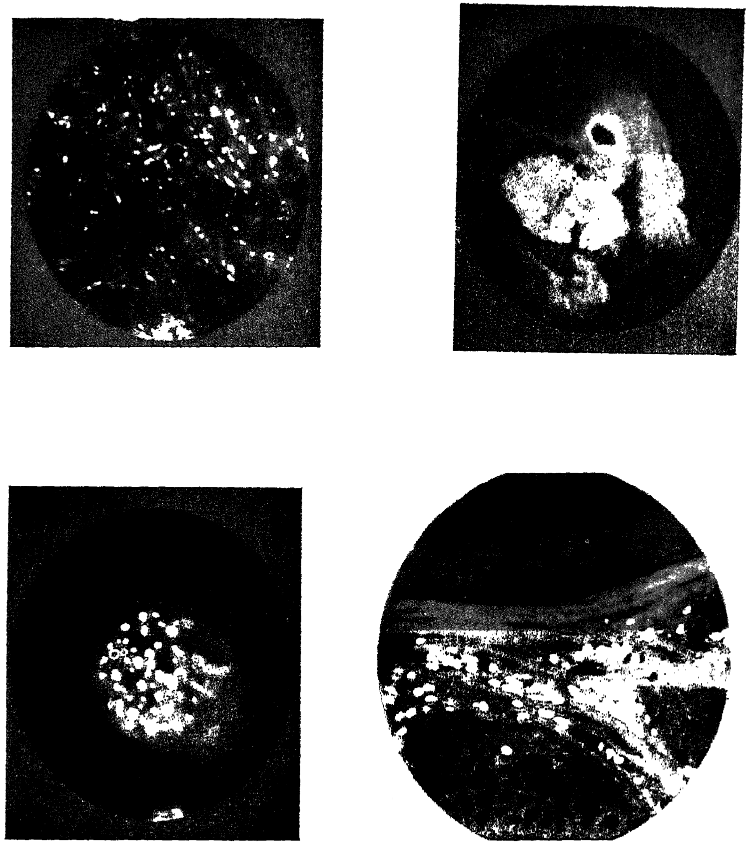
No. 20.—FLUORESCENCE MICROSCOPY OF THE BLACKBERRY (*Rubus fructicosus*) × 52.

Top—Photomicrograph in ordinary light. Orthochromatic plate; U.V. protective filter 1·2 mm.; exposure 60 secs.

Bottom—Fluorescence photomicrograph. Ultra-violet (carbon) arc, 6 amps.; orthochromatic plate; U.V. protective filter 1·2 mm.; exposure 4 mins.

(*See p.* 106.)

[*By courtesy of Messrs. E. Leitz.*



D

No. 21.—Palatal Vault.

A—Cell-nucleus in the spongy cell-tissue. Coloured green in ultra-violet light with berberine sulphate.

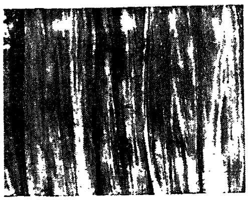
B—Effect of aurophosphine. The white portions are mucus in the mucus glands and are actually light green.

C—Section through nerve treated with thioflavin (oil-immersion lens). The individual threads are coloured bright blue in ultra-violet light.

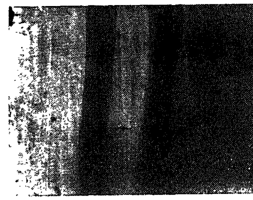
D—Fat-cells producing a bright blue fluorescence after treatment with chelidonium extract.

(*See p.* 244.)

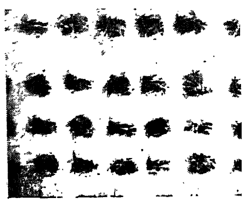
[Taken by M. Haitinger and H. Hamperl, and reproduced from the "Zeitschrift für mik. anatom. Forschung," by courtesy of Akademische Verlagsgesellschaft, M.B.H., Leipzig.



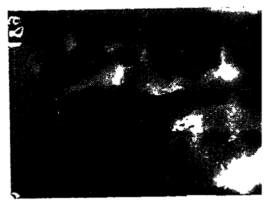
A



B



C



D

No. 22.—Textiles.

A—A piece of corset cloth photographed by ordinary light.

B—The same, in ultra-violet light, showing rayon effect threads.

C—Cotton fabric in ordinary light.

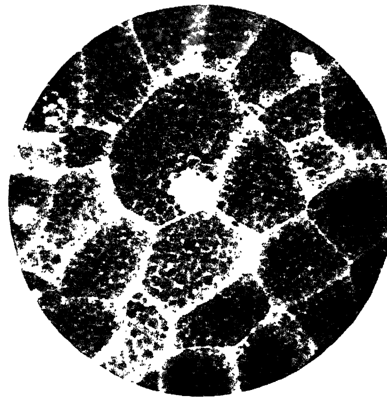
D—The same, in ultra-violet light, showing mildew.

Chance U.V. filter for lamp and Kodak Wratten 15A filter for camera. Ilford "soft gradation" plate (very fast). Mag. × 50. Exposure 1 hour.

(*See p.* 367.)

[*Taken by J. M. Preston.*

A



B

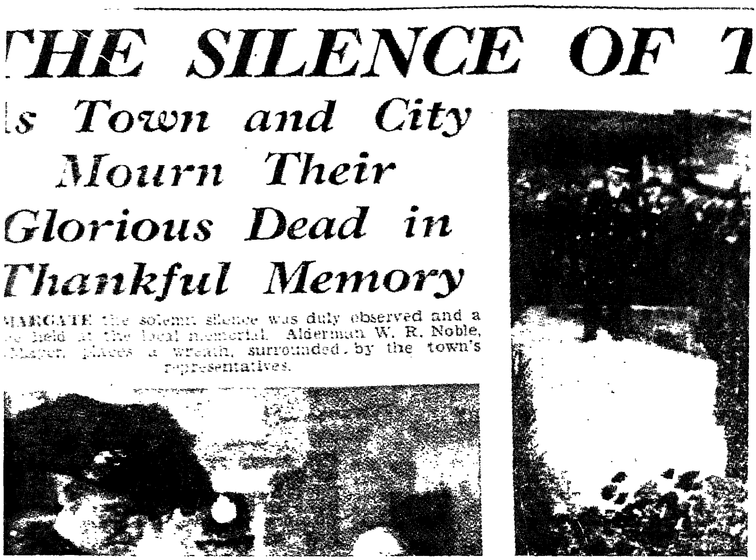
No. 23.—Seeds and Fruits.

A—Section of fruit of *Triticum* treated with 0·001 per cent. berberine sulphate and primuline yellow for 5 secs. Fruit and seed membrane fluoresce vivid yellow; cell wall of aleurone layer, blue; plasma, dull yellow; cell nucleus, golden-yellow; starch invisible. Exposure time, 2 minutes.

B—Section through the starchy endosperm of the seed of *Zea Mays*, treated with iodine solution and primuline yellow. Cell wall fluoresces yellow-green; plasma, blue; cell nucleus, white; starch, black. Exposure time, 6 minutes.

(*See pp.* 106 *and* 108.)

[Taken by M. Haitinger and L. Linsbauer, and reproduced from the "Beiheften zum Bot. Zentralblatt," 1935, by courtesy of Verlag C. Heinrich, Dresden.

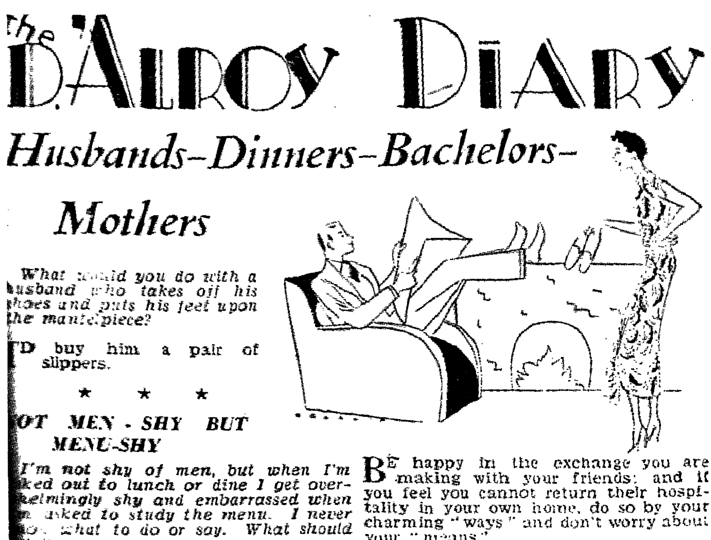


THE SILENCE OF T

s Town and City Mourn Their Glorious Dead in Thankful Memory

MARGATE the solemn silence was duly observed and a ... held at the local memorial. Alderman W. R. Noble, Mayor, places a wreath, surrounded by the town's representatives.

B



The D'Alroy Diary

Husbands-Dinners-Bachelors-Mothers

What would you do with a husband who takes off his shoes and puts his feet upon the mantelpiece?

I'D buy him a pair of slippers.

* * *

NOT MEN-SHY BUT MENU-SHY

I'm not shy of men, but when I'm asked out to lunch or dine I get overwhelmingly shy and embarrassed when asked to study the menu. I never know what to do or say. What should

BE happy in the exchange you are making with your friends: and if you feel you cannot return their hospitality in your own home, do so by your charming "ways" and don't worry about your "means."

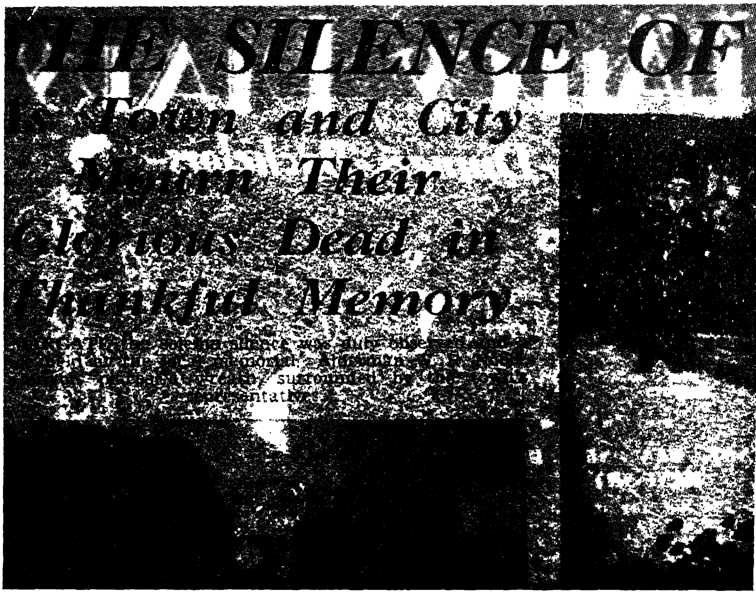
No. 24.—Penetration of Printing Ink. (*See also* No. 25.)

A—Photograph in ordinary light of newsprint showing defects on surface.

B—Photograph in ordinary light of the back of A. (*See also* No. 25.)

(*See p.* 348.)

[*Reproduced from "P.A.T.R.A. Journal," by courtesy of the Printing and Allied Trades Research Association.*

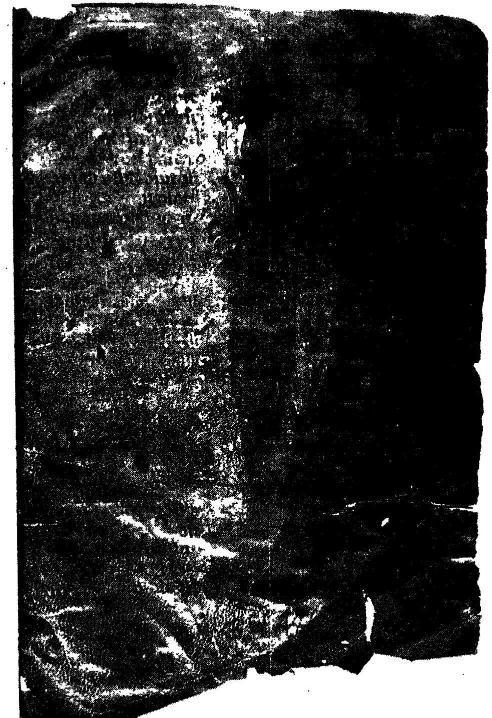


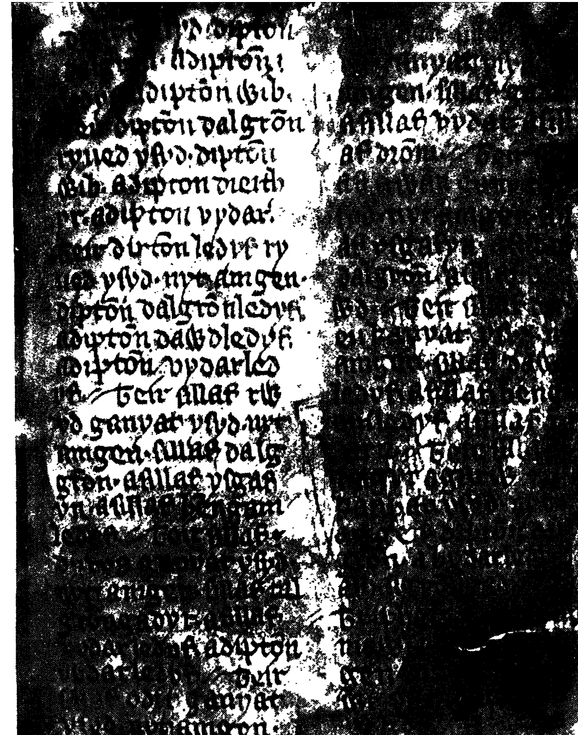
No. 25.—Penetration of Printing Ink. (*See also* No. 24.)

Photograph in ultra-violet light of the newsprint shown in No. 24 A; the fluorescence due to penetration of oil from the ink on the back (*see* No. 24 B) enables the latter to be seen through the sheet.

(*See p.* 348.)

[*Reproduced from "P.A.T.R.A. Journal," by courtesy of the Printing and Allied Trades Research Association.*





No. 26.—Faded Writing from the Peniarth MS. 20.

Left—Photograph in ordinary light.

Right—Photograph in ultra-violet light.

(*See p.* 279.)

[Photographs by the kindness of Mr. W. Ll. Davies, Librarian, The National Library of Wales. Reproduced from "Books and Documents," by courtesy of Messrs. Grafton & Co.

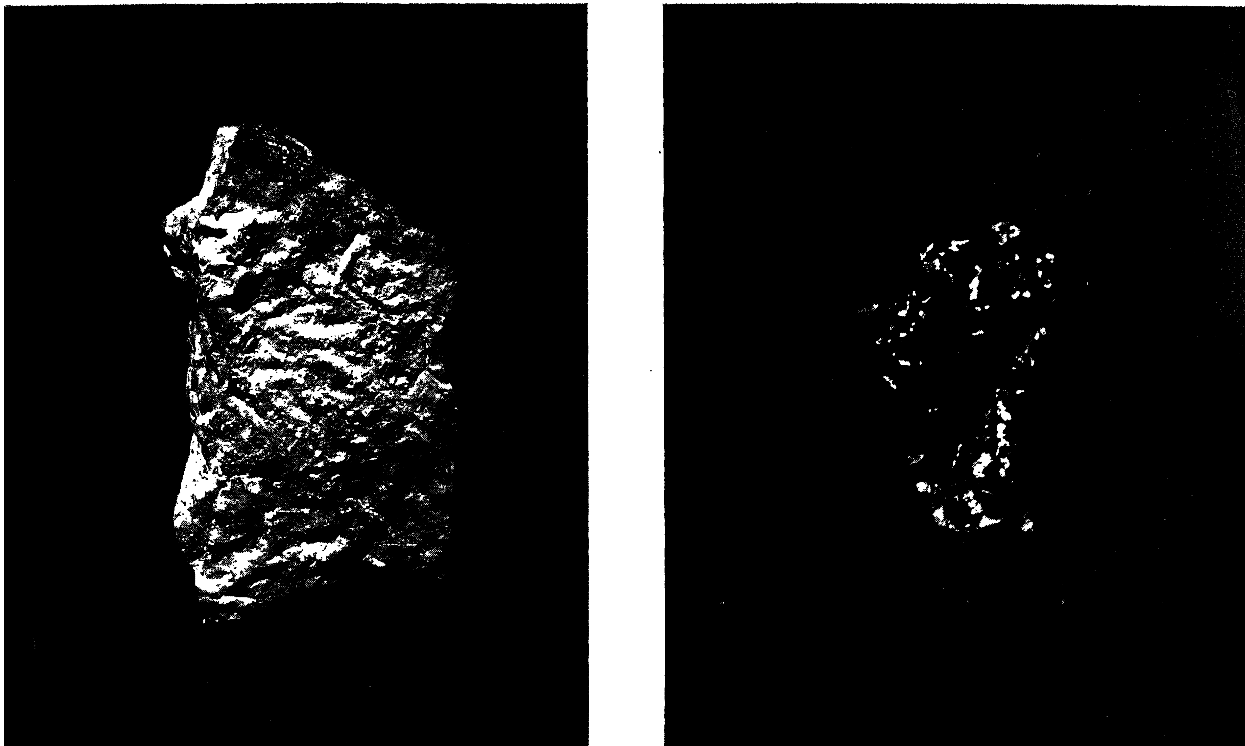
No. 27.—Diamond Chippings, × 52.

Top—Photomicrograph in ordinary light. Orthochromatic plate; exposure 15 secs.

Bottom—Fluorescence photomicrograph. Carbon arc; orthochromatic plate; exposure 5 mins.

(*See p.* 273.)

[*By courtesy of Messrs. E. Leitz.*



No. 28.—Autunite Disseminated in Porphyry.
(From Foley Mountain, S. Dakota.)
Left—Photograph in ordinary light.
Right—Photograph in ultra-violet light.

[By courtesy of Mr. W. M. Thornton, Jr.

SUBJECT INDEX.

(Principal References in heavy type.)

AUTHOR INDEX.